Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(1): 113-117
Received: 19-03-2015
Accepted: 17-04-2015

G. Jayapriya
PG & Research Department of Zoology, Voorhees College, Vellore – 632001, Tamil Nadu, India.

F. Gricilda Shoba
PG & Research Department of Zoology, Voorhees College, Vellore – 632001, Tamil Nadu, India.

Correspondence:
F. Gricilda Shoba
PG & Research Department of Zoology, Voorhees College, Vellore – 632001, Tamil Nadu, India.

GC-MS analysis of bio-active compounds in methanolic leaf extracts of *Justicia adhatoda* (Linn.)

G. Jayapriya, F. Gricilda Shoba

Abstract
Justicia adhatoda leaf extracts were prepared in methanolic solvent to study the phytochemical profile using Gas Chromatography - Mass Spectrometry method. GC-MS analysis of *J. adhatoda* leaf extracts revealed the existence of the major peaks presented in methanol were Amrinone (RT: 15.88); n-Hexadecanoic acid (RT: 16.33); Phytol (RT: 17.81); 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (RT: 18.04). From this study it is obvious that *J. adhatoda* leaf extracts contains many biologically active compounds and also it gives a detailed insight about the phytochemical profile which could be exploited for the development of plant based drugs and Insecticides.

Keywords: *Justicia adhatoda*, GC-MS, Methanol extracts, Phyto-compounds

1. Introduction
India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world [1]. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [2].
Justicia adhatoda is a species of plant in the Acanthaceae family. It is a shrub growing throughout India especially in the lower Himalayan regions. The name, *Justicia adhatoda* (Linn.) and *Adhatoda zeylanica* medic are used synonymously. It is commonly known as Vasaka or Malabar nut.
It is a highly valued Indian medicinal plant which is used in the treatment of respiratory diseases like asthma, cough, bronchitis and tuberculosis [3, 4]. The flowers, leaves and root have antispasmodic property. The activities against tuberculosis were reported by many researchers quite early [5, 6]. It has been used extensively as an important herbal drug in treating a wide variety of diseases and the leaves of the plant are the main source of drug formulation. For instance, the source of the drug 'vasaka' is well known in the indigenous system of medicine for its beneficial health effects, particularly in treating bronchitis [7]. The different parts of the plant is used in the Indian traditional medicine for the treatment of various diseases like asthma, joint pain, lumber pain and sprains, cough, eczema, malaria, rheumatism, swellings, venereal diseases [8, 9, 10].
In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [11, 12, 13].
Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. However, few reports are available with respect to the pharmacological properties of the plant. Keeping this in view, the present study has been undertaken to investigate the antibacterial effects and identify the phytoconstituents present in methanolic leaf extracts of *J. adhatoda* using GC-MS analysis.

2. Materials and Methods
2.1 Plant Collection and Authentication
The fresh leaves of *Justicia Adhatoda* (Linn.) of Acanthaceae family were collected from Jawadhu Hills, Vellore district of Tamilnadu, India and authenticated by professor P. Jayaraman, Botanist, Director, Plant anatomy research centre, Tambaram, Chennai, India in the month of May 2014 and registered Number of the Specimen is PARC/2014/2074.

2.2 Preparation of Extracts
Five hundred grams of coarse powder of shade dried leaves of *J. adhatoda* was extracted successively with methanol in soxhlet extractor for 48 hours. Dark green residues were obtained after concentrating the extract under reduced pressure (Yield 14.30%). The obtained extracts were stored in desiccators for further GC-MS and antimicrobial investigations. The plant extracts were diluted with respective solvents to the final concentration of 20 mg/ml. Microorganisms like *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Candida albicans* were used for testing.

2.3 Physicochemical Screening
2.3.1 Total Ash Content
Accurately weigh a quantity of the test sample, representing 2 to 4 g of the air-dried material, in a tarred crucible, and incinerate, gently at first, and gradually increase the temperature to 675 ± 25 ºC, until free from carbon, and determine the weight of the ash. If a carbon-free ash cannot be obtained in this way, extract the charred mass with hot water, collect the insoluble residue on an ash less filter paper, and incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness, and heat the whole to a temperature of 675 ± 25 ºC. If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15ml of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a temperature of 675 ± 25 ºC. Cool in desiccators, weigh the ash, and calculate the percentage of total ash from the weight of the drug taken.

2.3.2 Acid-Insoluble Ash
Boil the ash obtained as directed under Total Ash, above, with 25 ml of 3 N hydrochloric acid for 5 minutes, collect the insoluble matter on a tarred filtering crucible or ash-less filter, wash with hot water, ignite, and weigh. Determine the percentage of acid-insoluble ash calculated from the weight of drug taken.

2.3.3 Water-Soluble Ash
Boil the ash obtained as directed for Total Ash with 25 ml of water for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ash-less filter paper. Wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450 ºC. Subtract the weight of this residue, in mg, obtained under Total Ash, and calculate the percentage of water-soluble ash with reference to the weight of sample as determined under Total Ash.

2.3.4 Foreign Matter
Spread the sample out in a thin layer, and separate the foreign organic matter by hand as completely as possible. Weigh it, and determine the percentage of foreign organic matter in the weight of drug taken. Moisture content hot extraction method under alcohol soluble extractives, except to use water in place of alcohol and cold extraction method under alcohol soluble extractives, except to use water in place of alcohol.

2.4 Anti-Microbial Activity
2.4.1 Anti-bacterial Screening
Disc diffusion method was adopted for the antibacterial study [14, 15]. Ciprofloxacin at conc of 10mcg/disc was used as a standard. The filter paper impregnated with extracts (separately in each extracts at a concentration of 20 mgml-1) and ciprofloxacin disc were placed aseptically on agar medium which was already swabbed with the test organisms and incubated at 37 °C for 24h. The zone of inhibition in mm was measured.

2.4.2 Anti-fungal Screening
The antifungal activity of the crude extracts was determined against *Candida albicans* by disc diffusion method [14, 15]. Ketoconazole (10 mcgdisc-1) was used as standard. The filter paper disc impregnated with various extracts (20 mgml-1) individually and ketoconazole disc were placed aseptically on the sabouraud dextrose agar medium which was already swabbed with the test organism and incubated at 37 °C for24 h. The zone of inhibition (in mm) was measured and recorded.

2.5 GC-MS (Gas Chromatography-Mass Spectrometry) analysis
The phytochemical investigation of methanol extract of *J. adhatoda* leaf was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.:2.2, Thermo TSQ QUANTUM XLS Experimental conditions of GC-MS system were as follows: DB 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40 °C raised to 290 °C at 5 °C/min and injection volume was 1.0 µl. A scan interval of 0.5 seconds with scan range of 40-600 m/z. Total GC running time was 35min and the results were compared by using Wiley Spectral library search programme.

3. Results and Discussions
3.1 Physicochemical and Proximate Analysis
Physicochemical parameters and extractive value of *J. adhatoda* leaves were studied and results are given below. The moisture content was 18.20% (leaves) and the total ash content, acid insoluble ash, Water soluble ash and foreign matter values which were determined to be not more than 21.40%, 0.92%, 4.85%, 0.28% respectively. While study of extractive values can serve as a valuable source of information and provide suitable standards to determine the quality of plant material in future investigation. The proximate analysis value of the present data indicated that methanol (14.30%) extract showed higher extractives value when compared to other solvents.

3.2 Inhibition Activity on Micro-organisms
The methanol extracts of *J. adhatoda* were investigated for their potential anti-bacterial and anti-fungal activities. The extract was slightly affective for the *Escherichia coli*. Standard antibiotics ciprofloxacin (10mcg/disc) and ketoconazole (10mcg/disc) showed good inhibitory action on the micro-organisms tested. Methanolic extract indicated significant activity against *Staphylococcus aureus, Klebsiella pneumonia* and *Candida albicans*. *Pseudomonas aeruginosa* and *Proteus vulgaris* did not specify any antibacterial activities (Table 1.0).

3.3 Gas Chromatography Mass Spectrum (GCMS) Analysis
Phytochemical components in methonal extract of *J. adhatoda* by GC-MS report. The GC-MS analysis revealed the presence of 13 compounds (Table 2.0) from the methanol leaf extract of *J. adhatoda* (Figure 1). The major constituents were Amrinone (RT: 15.88) (Figure 1a); n- Hexadecanoic acid (RT: 16.33)

(Figure 1b); Phytol (RT: 17.81) (Figure 1c); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (RT: 18.04) (Figure 1d) along with other minor constituents were also present. The GC-MS chromatogram shows the peak area separation of the components. The above mentioned isolated compounds from the methanol extract of *J. adhatoda* leaves seem to posses the reported biological activity and further study of these phytoconstituents may prove the medicinal importance in future.

4. Conclusion

The correlation among the phytochemical constituents with their biological activities is now being the matter of innovative thought. *J. adhatoda* is a plant, traditionally used for the treatment of asthma, bronchitis, skin diseases, bio-insecticides etc. But till date, there are few reports on chromatographic analysis of methanolic extract of the plant. Here we report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plants. These identified phytoconstituents presumed to be responsible for eliciting the traditional activity of this plant *Justicia adhatoda*.

5. Acknowledgement

The authors are thankful to PG & Research Department of Zoology, Voorhees College, Vellore, Tamil Nadu, India for providing necessary facilities and support for the completion of this work.

6. Tables and Graphs

Table 1: Evaluation of antimicrobial activity of *J. adhatoda* (L.)

S. No.	Micro-organisms	Zone of inhibition (mm)	
		Std.	ME
1*	*Escherichia coli*	25	09
2*	*Klebsiella pneumonia*	24	18
3*	*Staphylococcus aureus*	30	20
4*	*Pseudomonas aeruginosa*	23	NS
5*	*Proteus vulgaris*	30	NS
6**	*Candida albicans*	22	14

***** *Indicates Bacterial Strains; ** Indicates Fungal strain; Std. – Standard; Me – Methanol extract; NS – Not specified.*

Table 2: Phytocomponents identified in the methanol leaf extracts of *J. adhatoda*

RT	Compound name	Molecular Formula	Area %
8.05	Eicosane, 2-cyclohexyl-	$C_{26}H_{52}$	2.65
12.01	Pentadecanoic Acid	$C_{15}H_{30}O_2$	9.11
12.39	Caryophyllene	$C_{15}H_{24}$	1.82
15.88	Amrinone	$C_{10}H_9N_3O$	11.12
15.98	Hexadecanoic acid, methyl ether	$C_{17}H_{34}O_2$	3.42
16.33	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	10.19
17.23	Ethyl 4-quinazoline -2-carboxylate	$C_{11}H_{10}N_2O_3$	6.48
17.68	9,12,15-Octadecatrienoic acid, methyl ether, (Z,Z,Z)-	$C_{19}H_{32}O_2$	2.99
17.81	Phytol	$C_{20}H_{40}O$	24.90
18.04	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	14.30
23.53	Squalene	$C_{30}H_{50}$	5.03
27.61	Stigmasterol	$C_{29}H_{48}O$	1.91
28.33	alpha-Sitosterol	$C_{29}H_{50}O$	6.08

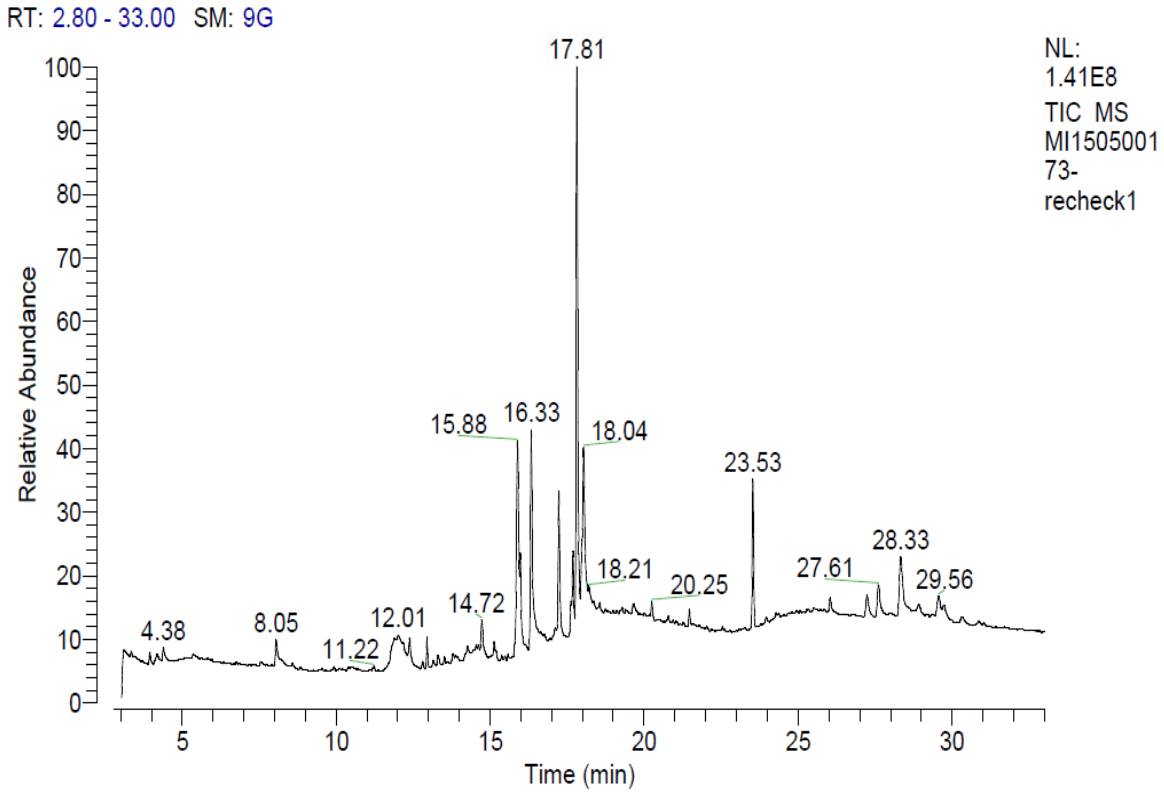

Fig 1: GC-MS Profile of Methanol leaf extract of *J. adhatoda*.

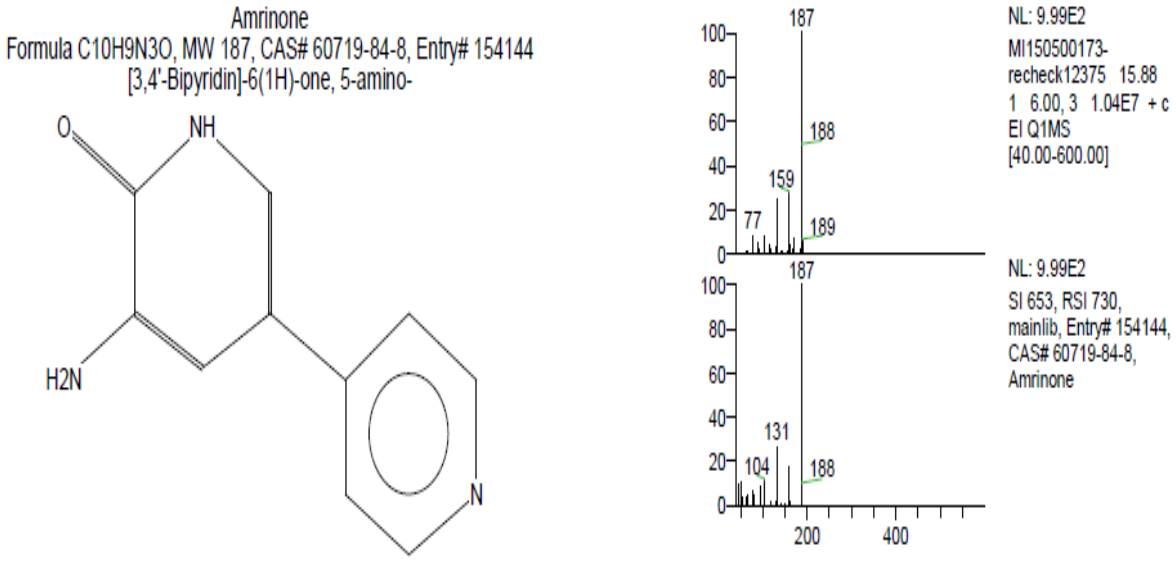

Fig 1a: Peak fragmentation of Chromatography Mass Spectrum (15.88)

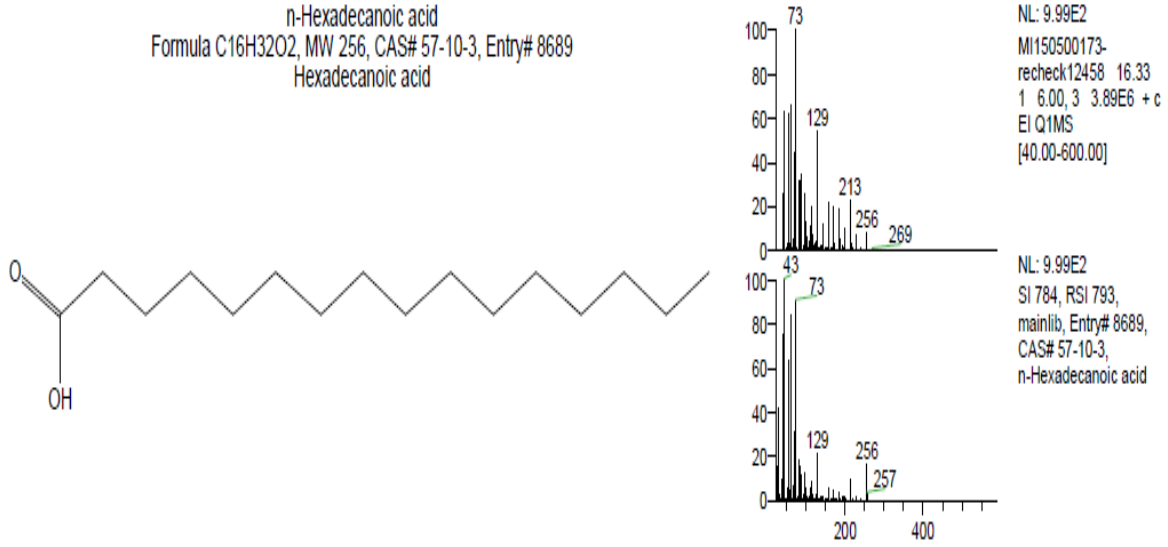

Fig 1b: Peak fragmentation of Chromatography Mass Spectrum (16.33)

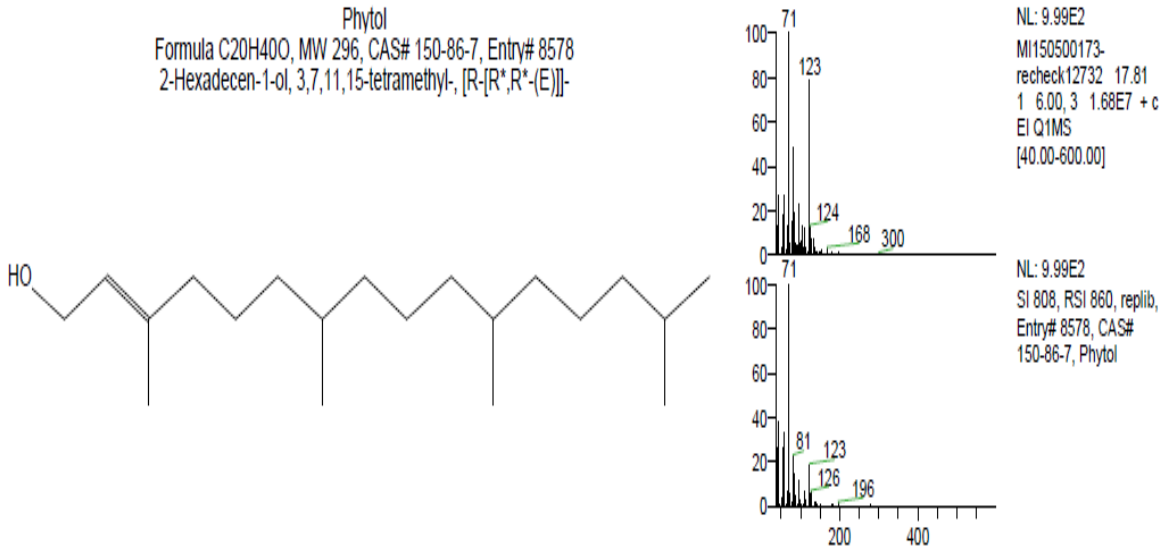

Fig 1c: Peak fragmentation of Chromatography Mass Spectrum (17.81)

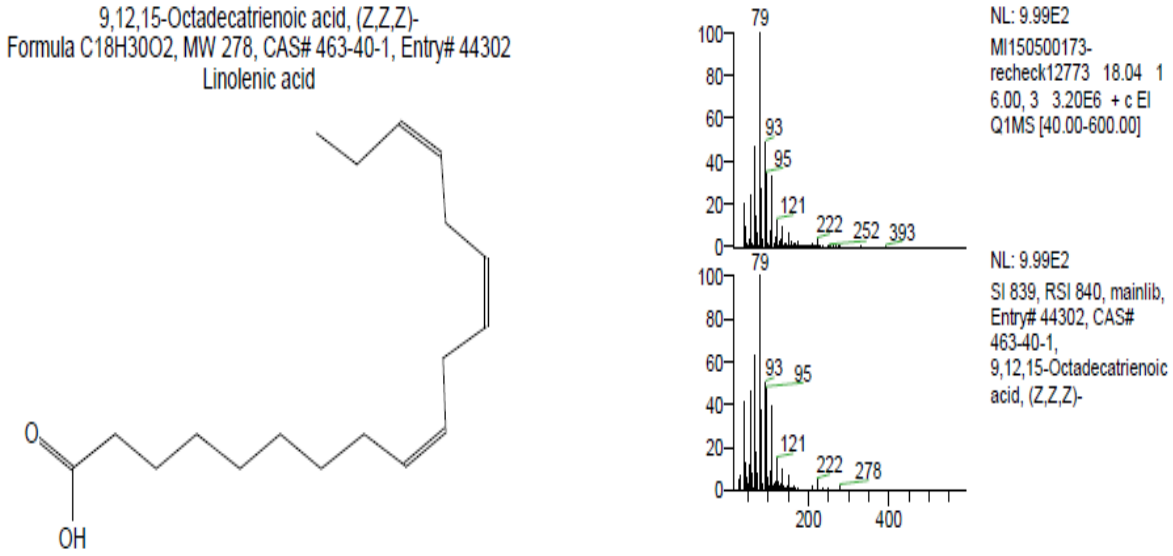

Fig 1d: Peak fragmentation of Chromatography Mass Spectrum (18.04)

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