



Research Article

EXPLORATION OF BIO-ACTIVE PHYTOCOMPONENTS AND EVALUATION OF ANTI-MICROBIAL POTENTIAL OF *STRYCHNOS POTATORUM*

Pitchiah Kumar Murugan ^{1*}, Kaliyamurthi Venkatachalam ²

¹ Part time Research Scholar, Centre for Research and Development, Prist University, Thanjavur, Tamil Nadu, India

² Assistant Professor, Department of Basic sciences, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India

*Corresponding Author Email: pitchiahkumar@yahoo.com

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ABSTRACT

Research interest towards herbal medicine grabs major attention in recent times due to its innumerable role in the process of drug discovery. It is evident that traditional medicines contribute as a therapeutic ailment for treating dreadful disease. Present investigation aimed at exploring the major phytochemicals present in the herb *Strychnos potatorum* (SP) and to screen its possible anti-microbial activity against selective pathogens. Ethanol (EESP), Aqueous (AESP) and Hydro alcoholic (HAESP) extracts were screened using biochemical, GCMS and FTIR analysis. Outcome of the study clearly signifies the presence of bioactive components such as alkaloids, flavonoids, tannins, phenols and saponin in the extracts. Results of GCMS justify the existence of ascorbic, oleic and erucic acid in EESP. Further higher level of active components was reported in EESP. FTIR study evidences the presence of major active functional groups in the extracts. Anti-microbial evaluation reveals that the EESP exhibits maximum zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumonia*. Present study provides an evidence-based data's with respect to the category and nature of phytochemicals present in the herb *Strychnos potatorum* and hence the promising anti-microbial activity may due to its biologically active phyto therapeutics which may render beneficial activity against infectious and cardiovascular diseases subjected to proper preclinical justification in near future.

Keywords: Herbal medicines, *Strychnos potatorum*, Phyto components, Anti-microbial, Oleic acid, GCMS, FTIR.

INTRODUCTION

Drugs derived from natural sources have significant contribution in the prevention and treatment of disease to mankind. In many developing countries, traditional and folklore medicines are the first drug of choice for their primary healthcare systems.^{1,2} Herbs are widely used and well explored in most of the traditional medicine and their curative potentials are well documented³.

World Health Organization estimates that nearly 80% of the people in developing countries rely on traditional remedies such as medicinal herbs and folklore medicines for their health care benefits. The use of medicinal plants has been developed over a long period of time and now plays a critical role in favorable health outcomes. Today, a number of pharmaceutical products currently approved by the Food and Drug Administration (FDA) have originated from plants; natural products (and their derivatives and analogs) represent over 50% of all drugs in clinical use.⁴⁻⁷

The number of multi-drug resistant microbial strains and the appearance of such strains had significantly reduced susceptibility to antibiotics are continuously increasing. This scenario has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent.⁸ Pharmaceutical industries are at dire need of phyto therapeutics with broad spectral anti-microbial property and offers minimal resistance when compare to conventional antibiotics.

Cardiovascular diseases (CVDs) become most prevalent cause of human morbidity and mortality globally. According to the research survey by global burden of disease Study it was estimated that nearly 29.6% of all deaths worldwide were caused by CVDs in 2010. It is estimated that the number of people that die from CVDs, mainly from heart disease and stroke, will increase to more than 24 million by 2030.⁹⁻¹²

Strychnos potatorum (SP) is a medium-sized, glabrous tree. Stem is fluted and covered with black, thick, square to rectangular scales¹³. Seeds of *Strychnos potatorum* possess diuretic, anti diarrheal and anti diabetic activity. Mannogalactans isolated from the seeds of *Strychnos potatorum* reported to have anti-hypercholesterolemic activity in experimental rats.¹⁴ Paste of seed has been consumed internally along with tender coconut milk in the management of urinary retention and other disorder¹⁵. As per the literature research it was strongly evident that the plant *Strychnos potatorum* has wide range of pharmacological activity which includes Anti-diabetic¹⁶, Anti-inflammatory^{17,18}, Anti-ulcerogenic¹⁹, Hepatoprotective²⁰, Antioxidant activity, Anti-arthritis²¹, Anti-nociceptive²², Anti-pyretic, Anti-diarrheal²³, Diuretics²⁴ and Antimicrobial properties²⁵.

Oxidative stress becomes major contributing factor for majority of the chronic diseases such as atherosclerosis, hematological and neurodegenerative disorders. Generation of free radicals due to oxidative stress factors associated with inflammation and other diseases became major health issues in recent years.^{26,27} Exploration of therapeutic lead from potential herbal source is highly essential for clinical management of several chronic

diseases. It has been already reported in our previous work that extract of EP possess significantly higher level of anti-oxidant property in the tested medium.²⁸ Hence in the present investigation aimed at evaluating the phytochemical and anti-microbial potential of the medicinal herb *Strychnos potatorum* using high throughput analytical techniques like GCMS and FTIR.

MATERIALS AND METHODS

Plant collection

Fruits of *Strychnos potatorum* were collected and were identified by expert taxonomist. Plant materials were then washed separately with fresh water to remove dirt and other contaminants. Epicarp of the fruits was separated and was shade-dried for several days with occasional sun drying. The dried materials were ground into coarse powder by a grinding machine and the materials were stored at room temperature for future use.

Preparation of the extract

About 1 kg of coarse powdered epicarp of *Strychnos potatorum* was passed through a 60 No mesh sieve. Air dried powdered drug was extracted with the following solvents like Water, Ethanol and mixture of Ethanol: water (6:4) (hydro-alcoholic extract) by using Soxhlet extraction. Then the extracts obtained such as aqueous extract of *Strychnos potatorum* (AESP), Ethanol extract of *Strychnos potatorum* (EESP) and Hydro-alcoholic extract of *Strychnos potatorum* (HAESP) was filtered, concentrated by rotary vacuum pump to get the solid mass.

Preliminary Qualitative Phytochemical Analysis

Qualitative biochemical analysis was carried for identification of category of phytochemicals in all three extracts. Presence of steroids identified using fluorescence in the presence of sulphuric acid. Alkaloids by using Mayer's test. Identification of flavonoids was carried out by ammonia test. Glycosides and triterpenoids using Borntrager's test and Liebermann-Burchard test. Ferric chloride and lead acetate test were used for identification of tannins and phenols, further presence of proteins and saponins identified using biuret and foam test.²⁹

Quantitative Estimation

Estimation of Total Flavonoid

Total flavonoid content in the extracts was determined using aluminum chloride method. In this method Quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. For this purpose, the calibration curve of quercetin was drawn. 1 ml of standard or extract solution was taken into 10 ml volumetric flask, containing 4 ml of distilled water. 0.3 ml of 5% NaNO₂ added to the flask. After 5 min, 0.3 ml 10% AlCl₃ was added to the mixture. At the 6th min add 2 ml of 1M NaOH was added and volume made up to 10 ml with distilled water. The absorbance was noted at 510 nm using UV-Visible spectrophotometer.³⁰

Estimation of Alkaloid

5 g of the test sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The

whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.³¹

Estimation of Tannin

The tannin content of the extracts was determined using Folin-Ciocalteu assay. Aliquot extract of the test drug of 100 µL was added to 750 µL of distilled water, 500 µL Folin-Ciocalteu reagents and 1000 µL of 35 % sodium carbonate (Na₂CO₃). The mixture was shaken vigorously after diluting to 10 mL of distilled water. The mixture was incubated for 30 min at room temperature and read at 725 nm. Distilled water was used as blank. Tannic acid standard solutions were prepared, and standard calibration curve was plotted with varying concentration. The total tannins content was expressed as Tannic acid mg/gm, as calculated from the prepared standard curve.³²

Determination of total Phenol content

The total phenol content of the extracts was determined using Folin-Ciocalteu reagents with analytical grade Gallic acid as the standard. 1 ml of sample was added to deionized water (10 ml) and Folin-Ciocalteu phenol reagents (1 ml). After 5 minutes, 20% sodium carbonate (2 ml) was added to the mixture. After being kept in total darkness for 1 hr, the absorbance was measured at 750 nm using a spectrophotometer. Amounts of total Phenol was calculated using Gallic acid calibration curve. The results were expressed as Gallic acid equivalents (GAE) mg/g of dry plant matter.³³

Fourier Transform – Infra Red Spectroscopy Study

Fourier Transform – Infra Red Spectroscopy Study (FTIR) IR data acquired with Shimadzu –IR affinity 1S portal, Software: Agilent Resolutions pro. About 20 mg of the test sample was taken on a micro spatula and grounded well with required quantity of KBr salt. Sample admixed with KBr with trituration aided by mortar and pestle until to get a uniform fine powder of sample-KBr mixture. Further mixture was loaded in pellet die and subjected to 5000-10,000 psi in pelletizer. Resulting pellet was placed in FTIR sample holder and expose to IR radiation to get the spectra.³⁴

GC-MS Analysis

GC-MS analysis of the extracts were carried out using Agilent 7890 B GC connected to 5977A MSD, NIST Ver.2.1 MS data library. GC-MS becomes a reliable tool for identifying the presence of volatile components including phytosterols in the herbal extracts and fractions.³⁵

Anti- Microbial Activity

Disc diffusion method adopted for antimicrobial profiling of the sample EESP at the concentration varying from 100 to 300 µg. MHB broth used for culturing specific target pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumonia* and standardized for the period of 24 h. Sub cultures of each strains inoculated in plate containing MHA medium. Test drug along with respective standards Streptomycin (20 µg) for anti-bacterial and Fluconazole (20 g) for anti-fungal injected on sterile disc positioned on each quadrant of the petri plates. Incubation allowed for the period of 24 h (bacterial) and 72 h (fungal). Inhibitory zone around the disc was measured and expressed in mm as an index of anti-microbial property.³⁶⁻³⁸

Table 1: Qualitative Phytochemical analysis of AESP, EESP and HAESP

Test Sample	Steroids	Alkaloids	Flavonoids	Glycosides	Terpenoid	Tannins	Poly phenol	Protein	Saponin
EESP	-	+	+	-	+	+	+	-	+
AESP	-	+	+	-	-	+	+	+	+
HAESP	-	+	+	-	-	+	+	+	+

Table 2: Qualitative Phytochemical analysis of AESP, EESP and HAESP

Phytoconstituents	AESP	EESP	HAESP
Total flavonoids (quercetin mg/gm)	0.77 ± 0.68	1.60 ± 0.24	1.09 ± 1.3
Total alkaloids (mg/gm)	0.69 ± 0.15	3.11 ± 0.26	1.13 ± 0.07
Total tannins (mg/gm) (Tannic acid mg/gm)	0.23 ± 0.08	0.52 ± 0.03	0.37 ± 0.02
Total Phenol (Gallic acid equivalents (GAE) mg/g)	0.016 ± 0.003	0.044 ± 0.06	0.028 ± 0.02

Mean with 3 replicates ± SD

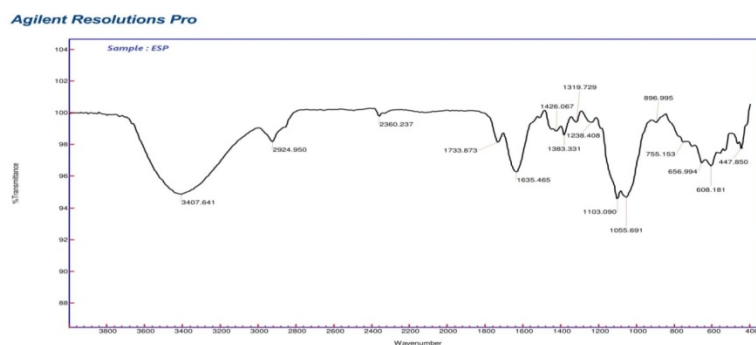


Figure 1: FT-IR Spectrum of ECP

Table 3: FT-IR Peak Table analysis of ECP

Absorption Peak No	Range	% Transmittance
1.	3407.64 cm ⁻¹	94.88
2.	2924.95 cm ⁻¹	98.19
3.	2360.23 cm ⁻¹	100.1
4.	1733.87 cm ⁻¹	98.16
5.	1635.46 cm ⁻¹	96.29
6.	1383.33 cm ⁻¹	98.61
7.	1238.40 cm ⁻¹	99.42
8.	1426.06 cm ⁻¹	98.86
9.	1319.72 cm ⁻¹	99.42
10.	1103.09 cm ⁻¹	94.59
11.	1055.69 cm ⁻¹	94.69
12.	896.99 cm ⁻¹	99.40
13.	755.15 cm ⁻¹	98.17
14.	656.99 cm ⁻¹	96.85
15.	608.18 cm ⁻¹	96.67
16.	447.85 cm ⁻¹	97.78

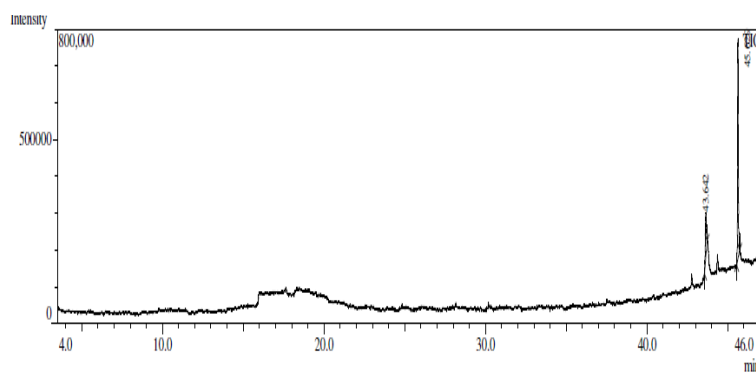


Figure 2: GCMS Chromatogram of AESP

Table 4: GCMS Peak Table analysis of AESP

Peak #	R. Time	Area %	Compound Name
1	43.64	23.30	Silane
2	45.62	76.70	Tetrapentacontane
		100.00	

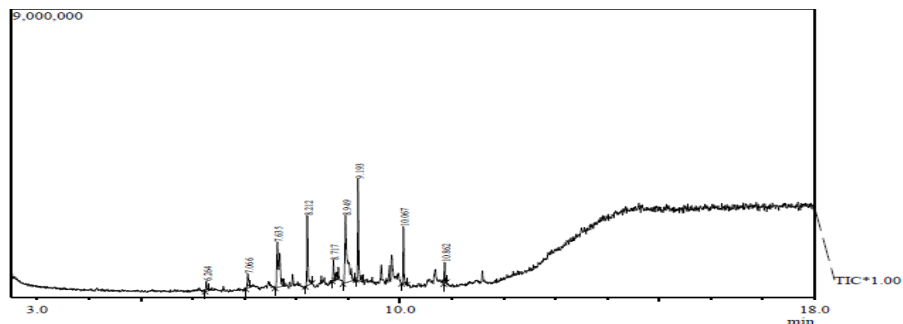


Figure 3: GCMS Chromatogram of HAESP

Table 5: GCMS Peak Table analysis of HAESP

Peak #	R. Time	Area %	Compound Name
1	6.26	2.08	Decane, 2,3,5,8- tetramethyl-
2	7.06	3.74	1-Iodo-2-methyundecane
3	7.63	20.05	Phenol,3,5- bis (1,1-dimethylethyl)-
4	8.21	13.79	2,3- Di hydrooxazole
5	8.71	3.11	Eicosane
6	8.94	29.30	Decanoic acid
7	9.19	16.43	Capric Acid
8	10.06	8.04	Heptacosane
9	10.86	3.45	Octacosane
		100.00	

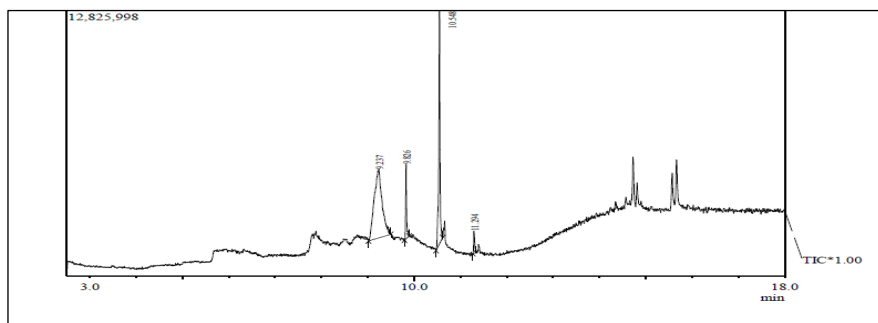


Figure 4: GCMS Chromatogram of EESP

Table 6: GCMS Peak Table analysis of EESP

Peak #	R. Time	Area %	Compound Name
1	9.23	59.75	3-Methylmannoside
2	9.82	7.30	Ascorbic acid
3	10.54	30.42	Oleic acid
4	11.29	2.53	Erucic acid
		100.00	

Table 7: Zone of Inhibition data on Anti-bacterial activity of EESP against selected Bacterial strains

Sample code	Zone of inhibition (mm)																	
	<i>Bacillus subtilis</i>			<i>Staphylococcus aureus</i>			<i>Enterococcus faecalis</i>			<i>Pseudomonas aeruginosa</i>			<i>Escherichia coli</i>			<i>Klebsiella pneumoniae</i>		
	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg
EESP	8	10	16	10	12	18	-	7	13	-	-	8	8	11	16	-	-	7
Streptomycin (20 µg)	25			26			18			12			23			19		

- = Not active

Table 8: Zone of Inhibition data on Anti-Fungal activity of EESP against selected fungal strain

Sample code	<i>Candida albicans</i>		
EESP	100 µg	200 µg	300 µg
	11	12	16
Fluconazole (20 µg)	20		

RESULTS

Qualitative Phytochemical analysis of AESP, EESP and HAESP

The results of the preliminary qualitative phytochemical analysis of AESP, HAESP and EESP justify the presence of alkaloids, flavonoids, tannins, phenols and saponin. In this presence of terpenoids was only evident in the EESP which may be unique for EESP for its expected biological activity. The results were tabulated in Table 1.

Quantitative Phytochemical analysis of AESP, EESP and HAESP

The quantitative estimation of total flavonoid, total alkaloids, total tannins and total phenols in AESP were found to be 0.77, 0.69, 0.23 and 0.016 mg/gm respectively, For EESP, the values were found to be 1.60, 3.11, 0.52 and 0.044 mg/gm. The quantitative estimation of HAESP for of total flavonoid, total alkaloids, total tannins and total phenols were found to be 1.09, 1.13, 0.37 and 0.028 mg/gm respectively. The results were tabulated in Table 2.

FT-IR Analysis of EESP

Strong intense peak at 3407 cm^{-1} may be due to broad O-H stretching vibration. Medium peaks at 2924 cm^{-1} may be due to presence of N-H str. less intense peak at 2360 cm^{-1} due to the presence of O-H and N-H stretching vibration. Peak at 1733 cm^{-1} due to the presence of Amide ($\text{C}=\text{O}$ stretching vibration) and peak at 1635 cm^{-1} due to $\text{C}=\text{C}$ stretching vibration; peak at 1383 cm^{-1} due to N-O stretching vibration and peak at 1426 cm^{-1} due to C-H deformation vibration. Peak at 1103 cm^{-1} due to the presence of C-O stretching vibration and at 896 cm^{-1} may be due to N-H out-of-plane bending vibrations. Sharp peak at 656 cm^{-1} due to $\text{C}=\text{O}$ (conjugated) deformation vibration and at 447 cm^{-1} may be due to C-S stretching vibration. The results were tabulated in Table 3 and illustrated in Figure 1.

GCMS analysis of AESP, EESP and HAESP

GCMS analysis of the sample AESP reveals the presence of two independent peaks, whereas for HAESP it revealed the presence of nine prominent peaks and for EESP it shown 4 prominent peaks. The peak 2, 3 and 4 of EESP corresponds to the presence of ascorbic, oleic and erucic acid. The results were tabulated in Table 4 to 6 and illustrated in Figure 2 to 4.

Anti-Microbial Profiling of EESP

EESP has revealed maximum zone of inhibition against *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumonia* with the inhibitory zone of 16, 13 and 7 mm at similar concentration. As shown in Table 7. Anti-fungal activity of the extract EESP has revealed concentration dependent activity against *Candida albicans* with the maximum zone of 16 mm at the concentration of 300 µg when compared to that of the standard fluconazole. As shown in Table 8.

DISCUSSION

Medicinal herbs are capable of synthesizing secondary metabolites³⁹ which can be used for the new drugs discovery and development. Medicinal plant extracts are potential source for the development of new agents effective against many infections disease that are currently difficult to treat. The results of the preliminary phytochemical analysis of the sample AESP, HAESP and EESP reveal the presence of bioactive phyto components such as alkaloids, flavonoids, tannins, phenols and saponin. In this presence of terpenoids was only evident in the EESP which may be unique for EESP for its expected biological activity.

The biomedical potential of the herbs possesses some biologically active components that produce a definite physiological action on the human health. The most important bioactive substances are alkaloid, tannin, flavonoid and phenolic compounds.⁴⁰ In the present study out of three extracts subjected for analysis the EESP has shown increased level of secondary metabolites when compared to aqueous and hydro alcoholic extracts. The quantitative estimation of total flavonoid, total alkaloids, total tannins and total phenols in EESP were found to be 1.60, 3.11, 0.52 and 0.044 mg/gm.

Terpenoids are considerably high potential phyto components which plays very vital role and being the major component of essential oils that make them valuable in the perfumery industry. Within the food industry, the flavoring and preservative potential of essential oils have been well understood along with their general antimicrobial and antioxidant effects.⁴¹⁻⁴³ Saponins are other group of secondary metabolites found in most of the medicinal herbs. It was evident though several studies that plant saponin are possess potential anti-obese activities. Saponin could increase fatty acid oxidation and inhibit hepatic glucose production thereby lowering plasma FFA levels.⁴⁴ Plant poly phenols are potential scavengers of free radicals in general. It was well proven fact that regular dietary supplementation of poly phenols aid in prevention of pathological diseases and for the improvement of human health conditions.⁴⁵ In the present study FT-IR analysis of the sample ESP reveals the presence of most significant functional groups such as N-H, O-H, $\text{C}=\text{O}$ etc.

About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer.⁴⁶ Recent trends, however, show that the discovery rate of active novel chemical entities is declining.⁴⁷ Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethno medicinal plants in India.⁴⁸⁻⁵⁰ It was observed from the GCMS analysis of the present investigation that the bioactive component like ascorbic and oleic acid was present exclusively on the EESP. Further it was evident through research that oleic acid, improve lipid profile and⁵¹, maintain a balance of body weight⁵² and prevent palmitate-induced mitochondrial dysfunction, insulin resistance and inflammatory signaling in neuronal cells⁵³ and skeletal muscle⁵⁴.

CONCLUSION

Strychnos potatorum is a potential medicinal herb which exhibit versatile pharmacological action due to diverse phyto therapeutics present in it. From the results of the present investigation it was concluded that the EESP has significantly higher proportion of phyto components when compare to aqueous and hydro alcoholic extracts. GCMS reveals the presence of ascorbic and oleic acid in EESP. Anti-microbial property of EESP may be due to presence of biologically active phyto components such as alkaloids, flavonoids, tannins, phenols and saponin. Further presence of oleic acid justifies the folklore claim of this herb against cardiovascular and inflammatory disorders. Preclinical investigation will be carried out in future to study its mechanism in suitable animal model.

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