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Research Article

Extraction Isolation Phytochemical Screening and Analysis of Madhunashini (*Gymnema Sylvestre*)

Swati Wakchoure, Akash Nalawade, Tejal Dingore*, Dev Gaikwad, Leena Gharat, Kamini Ghavat

Shivajirao S Jondhle College of Pharmacy, Asangaon, Thane.

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ABSTRACT

The present study focuses on the extraction, isolation, phytochemical screening, and analysis of Madhunashini (*Gymnema Sylvestre*), a medicinal plant renowned for its antidiabetic and therapeutic properties in traditional Ayurvedic medicine. The crude extracts of powdered leaves were then analyzed through preliminary phytochemical screening, revealing the presence of key secondary metabolites such as alkaloids, flavonoids, saponins, glycosides. Further isolation of bioactive components was carried out using chromatographic techniques, and the compounds were characterized using analytical tools. By applying various phytochemical and analytical techniques, the research seeks to validate the chemical basis of the plant's medicinal value and provide insights for its potential use in phytopharmaceutical development.

INTRODUCTION

Medicinal plants, which form the backbone of traditional medicine, have been subject for very intense pharmacological studies for last few decades; this has been brought about by the acknowledgment of plants as potential sources of new compounds of therapeutics value and as sources of lead compounds in the drug development. In developing countries, it is estimated as 80% of the population depend on traditional medicine for their primary health care. Thus, need for screening of medicinal plants for bioactive compounds arises as a basis for further pharmacological studies.[1]

Gymnema Sylvestre (G. sylvestre) (retz.) schult.[2] belonging to family asclepiadaceae is widely found in many different parts of the world. G. sylvestre mainly native to Asia (including the Arabian Peninsula), Africa and Australia, the Deccan peninsula of western India, tropical Africa, Malaysia, Srilanka, Japan, Germany, southern China, Vietnam and USA. G. sylvestre is well

***Corresponding Author:** Tejal Dingore
Address: *Shivajirao S Jondhle College of Pharmacy, Asangaon, Thane.*
Email : tejald070@gmail.com

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known for its sweet taste suppressing activity and also used for the treatment of diabetes mellitus and obesity. In traditional medicine G. sylvestre is used as a diuretic and remedy for diabetes mellitus.[3,4]

Gymnema Sylvestre has a long history of use in traditional medicine, particularly in Ayurvedic systems. The plant, known as "Gurmar" in Sanskrit, means "sugar destroyer"[5,6,7] and is used to support diabetes management, reduce sugar cravings, and improve digestive health. Modern research has explored the bioactive compounds, including gymnemic acids, to understand the mechanisms behind its taste-modifying and anti-diabetic effects. In recent times, researchers have investigated gymnemic acids and other bioactive compounds to better understand its effects.[8,9,10,11,12]

MATERIAL AND METHODS

Materials and Methods outlines the experimental design, procedures, and analytical techniques used in the study.

Collection and Authentication of Plant:

The powdered leaves of *Gymnema Sylvestre* were obtained from Trivikram Products. The leaves were initially dried under shade, away from direct sunlight, before being processed into powder. The dried leaves were then cleaned using a mechanical grinder to remove any extraneous matter and coarsely ground. The resulting powder was passed through a 120-mesh sieve to ensure uniformity and remove any excessively fine particles. The retained coarse powder was then utilized for subsequent extraction procedures. Dr. Harshad Pandit performed authentication of the plant material through morphological comparison. A voucher specimen has been deposited.

Figure 1: Authentication of Plant *Gymnema Sylvestre*

Pharmacognostic Studies:

1. **Macroscopy**

Visual examinations of leaves were conducted by the naked eye, and the characteristics such as shape, colour, taste, and smell of the leaves were assessed and documented.

2. **Microscopy:**

Morphology of fresh *Gymnema Sylvestre* was studied. Microchemical and pulverized

characteristic of fresh leaves was taken for atomic evaluation.

Evaluation Of Physical Constant:

1. **Determination of Foreign Organic Matter:**

5 grams of *Gymnema Sylvestre* leaf powder were weighed and spread on a clean, white surface under proper lighting. `Foreign organic matter were manually separated using forceps. The separated foreign matter was accurately weighed. The percentage of foreign organic matter was calculated.

2. **Determination of Moisture Content:**

Five grams of *Gymnema Sylvestre* powder were accurately weighed using an analytical balance and transferred to a pre-tared porcelain crucible. The crucible containing the sample was then placed in a preheated oven maintained at 105°C for a duration of 10 to 15 minutes. Following the drying period, the crucible was carefully removed from the oven and allowed to cool to room temperature in a desiccator, ensuring minimal moisture reabsorption. Subsequently, the crucible and dried sample were re-weighed using the same analytical balance. The moisture content was calculated as the percentage weight loss relative to the initial sample weight.

3. **Determination of Total Ash**

Weigh an empty crucible and record its weight. Add 5 g of the sample to the crucible and weigh again. Place the crucible in a muffle furnace and incinerate at 550–600°C for 2–3 hours until the sample is completely ashed. Cool the crucible in a desiccator and weigh it along with the ash. The total ash content is calculated as a percentage of the initial sample weight.

4. **Determination of Water-Soluble Ash**

Weigh the total ash obtained from the previous step and add 10–20 mL of distilled water. Stir the mixture thoroughly and filter it through a pre-weighed filter paper. Dry the residue retained on the filter paper, weigh it, and determine the water-soluble ash by subtracting the residue weight from the total ash.

5. **Determination of Water-Insoluble Ash**

Follow the same procedure as water-soluble ash, but instead of calculating the dissolved portion, the weight of the residue retained on the filter paper is recorded as water-insoluble ash.13,14

Extraction Of *Gymnema Sylvestre*

50 grams of *Gymnema Sylvestre* powder were subjected to maceration in 500 mL of 40% v/v ethanol for 100 hours at ambient temperature. Following the maceration period, the resulting extract was separated from the marc by filtration through [specify filter type, e.g., Whatman No. 1 filter paper]. The filtrate was then concentrated by evaporation under controlled conditions on a temperature-regulated hot plate until a dry, solid extract was obtained. This extract was subsequently pulverized to a fine powder and stored for further analysis.

Preliminary Phytochemical Tests:

Table 1: Preliminary Phytochemical tests 15,16

Sr. No.	Constituents	Test
1	Saponin	Foam Test : Shake extract with water vigorously in a test tube.
2	Alkaloid	Wagner's Reagent : Take 2 mL of aqueous extract in a test tube. Add a few drops of Wagner's reagent.
3	Flavonoid	Lead Acetate Test : Add few drops of lead acetate solution to the extract.

4	Tannin	Gelatine Test : Take 2 mL of aqueous extract in a test tube. Add a few drops of 1% Gelatine solution.
5	Carboxylic acid	Sodium Bicarbonate Test : Add a pinch of $NaHCO_3$ to the extract.
6	Triterpenoid	Salkowski Test : Mix extract with chloroform, then add concentrated H_2SO_4 carefully along the side of the test tube.

Analytical Studies

A. Thin Layer Chromatography

1. Plate Preparation: Pre-coated silica gel TLC plate; sample spotted 1 cm from the base.
2. Mobile Phase: Chloroform: Methanol (9:1 v/v) prepared and chamber saturated.
3. Development: Plate placed in the saturated chamber; solvent allowed to ascend.
4. Plate Removal & Drying: Plate removed at solvent front; dried.
5. Visualization: Detecting reagent sprayed; spots observed under UV light/iodine.

B. UV Spectroscopy

1. Prepare the alcoholic extract of the drug.
2. Dissolve 1 mg of extract in 1 mL of methanol/ethanol to prepare a stock solution of 10 mg/ml.
3. Take 1 mL of the stock solution and dilute it to 10 mL with methanol/ethanol (final concentration = 100 µg/mL).
4. Scan the prepared sample solution in the wavelength range of 200-400 nm using UV spectroscopy.
5. Use methanol/ethanol as a blank.
6. Record the absorbance spectrum and identify the λmax (wavelength at maximum absorption).

C. Fourier Transform Infrared Spectroscopy

1. Dried poodles of crude drug extract were used for FTIR analysis.
2. The sample was loaded onto FTIR spectroscope (BROKER, ALPHA II, Platinum AIR (in a scan range 350-8000 cm^-1)

D. GS-MS (Gas Chromatography-Mass Spectroscopy)

1. To determine the various bioactive chemicals, present, the extract was submitted to GC MS analysis.
2. The Agilent Technologies 7890 B GC system instrument used software chemstation to analyse the material.
3. The DBT-S-MS column, which measured 30 m x 0.15 m x 0.5 m, was utilized.

Three microliters of methanol extract were injected into the specimen at a temperature of 250°C at a steady rate with a split ratio of 10:1.

Oven temperature and carrier gas as helium delay of three minutes Start at 40°C for three minutes, then ramp up to 320°C at a rate of 1°C per minute, holding for fifteen minutes.[17,18,19]

RESULTS AND DISCUSSION

Pharmacognostic Evaluation:

In pharmacognostic study of leaves of *Gymnema Sylvestre* macroscopic, microscopy, powder characteristics and physicochemical parameters were studied.

1. Macroscopy

Figure 2: Macroscopy Of *Gymnema Sylvestre* leaf

Table 2: Morphological and Organoleptic

Sr. No.	Parameters	Features
1	Colour	Green
2	Odor	Characteristic
3	Taste	Bitter and Astringent
4	Size	1.25 -2.0 in X 0.5-1.25 in
5	Shape	Ovate, Elliptic

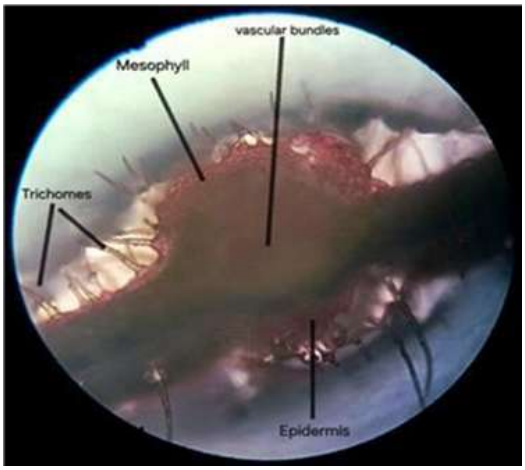


Figure 3: Transverse section of *Gymnema Sylvestre*

Microscopic study of leaves part:

Table 3: Powder Characteristics of *Gymnema Sylvestre* leaves

Sr. No.	Reagents	Observation	Characteristics
1.	Phloroglucinol + Conc. HCl	Pink Colour	Fibers and vessels
2.	Sudan red	Red colour	Trichomes, Epidermal cells, Vascular bundles and Mesophyll

1. **Trichomes**
   Unicellular or multicellular non-glandular trichomes
2. **Epidermal Cells**
   The upper and lower epidermal layers, with polygonal cells.
3. **Vascular bundles**
   Collateral and closed
4. **Fibers**
   Lignified fibers and spiral/thickened xylem vessels.
5. **Mesophyll**
   Dorsiventral, shows two distinct layers.

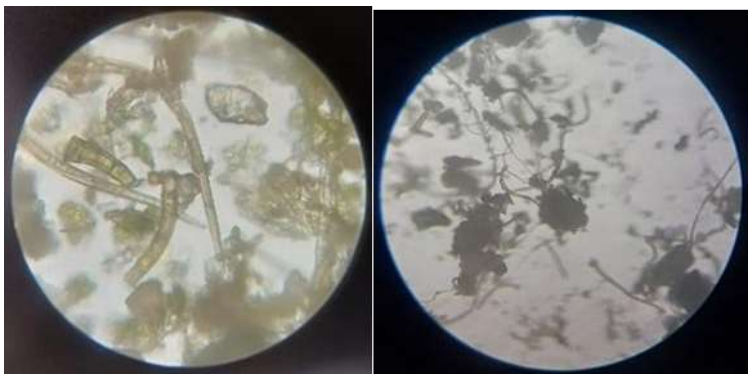


Figure 4: Powder characteristics of *Gymnema Sylvestre*

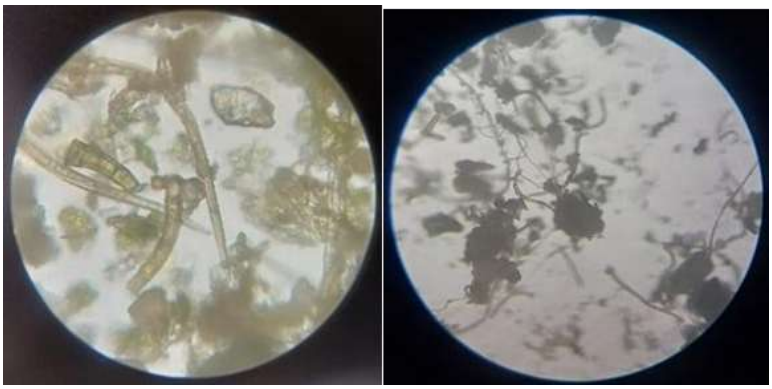


Figure 5: Lower epidermis of *Gymnema Sylvestre*

Determination of Physical Constants:

1. Determination of Foreign Organic Matter:

No Foreign Organic Matter detected.

2. Determination of Moisture Content:

Table 4: Loss of moisture obtained

Time	Loss of Moisture (w/w %)
00	0.0
01	1.6
02	3.2
03	4.8

3. Determination of Ash values:

Table 5: Ash values obtained

Sr. No.	Parameters	Value (w/w)
1.	Total Ash	4.86
2.	Water Soluble Ash	2.50
3.	Water Insoluble Ash	2.36

4. Extractive Values

Table 6: Extractive value obtained

Parameters	Value (w/w%)	Color	Appearance	Yield
Alcohol Soluble	11.8	Dark Brown	Semi-solid and sticky	5.90

Preliminary Phytochemical Study

Qualitative analysis was done to detect various chemical constituents by performing tests for alkaloids, glycosides, Saponin, flavonoids, tannins, carboxylic acid and triterpenoids.

Table 7: Preliminary phytochemical screening of extract

Sr. No.	Constituents	Result
1.	Saponin	+
2.	Alkaloid	+
3.	Flavonoids	+
4.	Tannins	-
5.	Carboxylic acid	+

6.	Glycosides	+
7.	Triterpenoid	-

Note: '+ve' used for positive test and '-ve' used for negative test.

The results of preliminary phytochemical study shown in Table No. presence of Saponin, Alkaloid, Flavonoids, Carboxylic acids and Glycosides.

Analytical Study

1. TLC (Thin Layer Chromatography)

Thin layer chromatography technique is used for the separation, isolation and identification of constituents presents in the Ethanol extract.

Table 8: Thin Layer Chromatography values obtained

Extract	Solvent System	Detecting agent	Color of Spot	Rf Value
Ethanoic Extract	Chloroform : Ethanol 9:1	Sulfuric acid	Yellow	0.28
			Green	0.54

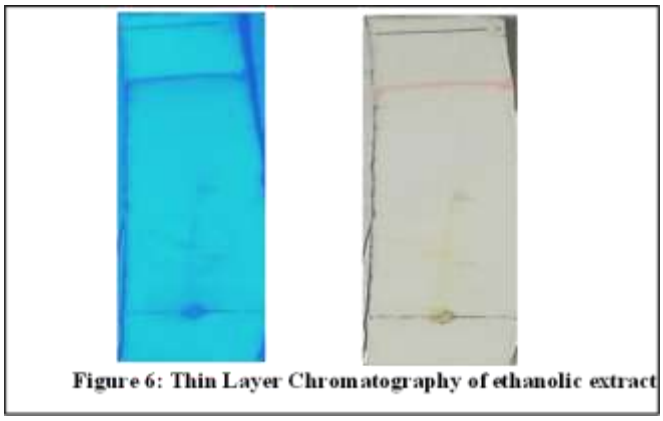


Figure 6: Thin Layer Chromatography of ethanolic extract

2. UV Spectroscopy

Figure 7: UV spectra of ethanolic extract

Table 9: UV spectra of ethanolic extract

Observation	Result
Peak Spotted	210 to 290 nm

3. FTIR (Fourier Infrared)

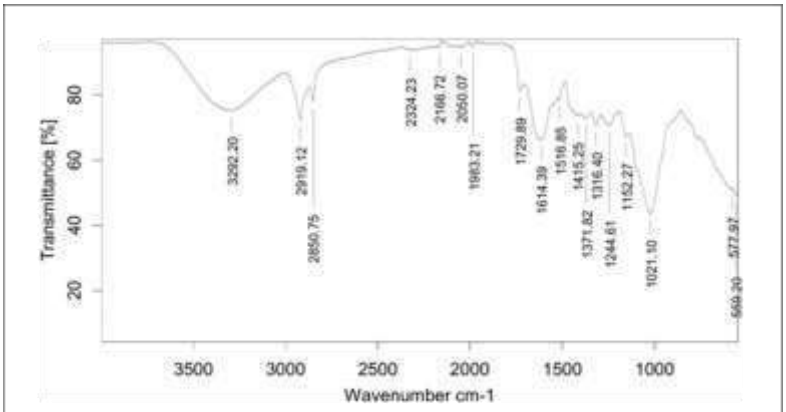


Figure 8: FTIR of *Gymnema sylvestre* leaves powder

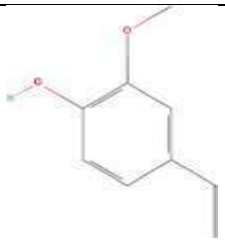
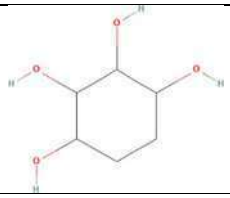
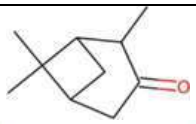
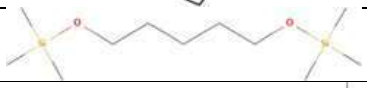
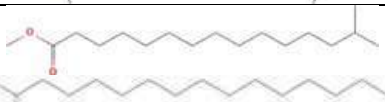
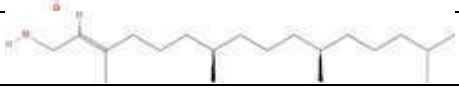
Table 10: FTIR results for *Gymnema Sylvestre*

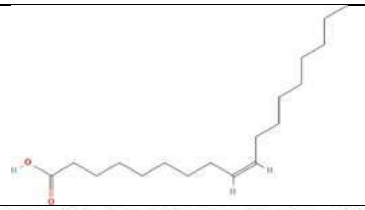
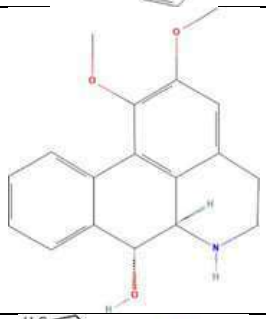
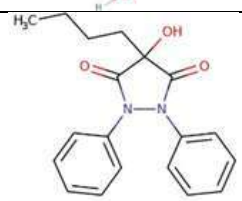
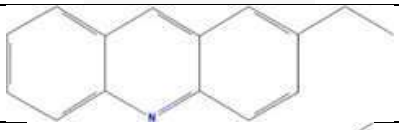
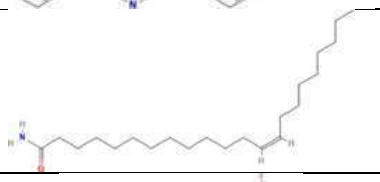
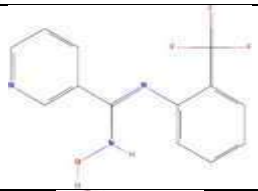
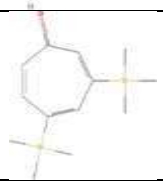
Wavenumber	Absolute Intensity	Width	Bond	Functional Group
3292.2016	0.753	429.9624	O-H stretch, H- bonded	Alcohols, Phenols
2919.1199	0.722	133.2092	C-H stretch	Alkane
2850.7524	0.780	13.9105	C-H stretch	Alkane
2324.2251	0.934	3383.1935	C≡N stretch (nitrile	Nitrile
2166.7181	0.943	572.6755	C≡C stretch	Alkyne
2050.0719	0.945	112.7555	Overtone/combination n band or highly conjugated system	-
1983.2124	0.947	14.1838	Overtone/combination n band or highly conjugated system	-
1729.8855	0.813	848.7900	C=O stretch	Carbonyl (aldehyde, ketone, ester, carboxylic acid)
1614.3919	0.663	131.3951	C=C stretch or C-C stretch	Alkene or aromatic ring
1516.8542	0.784	381.8738	N-H bend or C-C stretch	1° amine or aromatic ring
1415.2451	0.735	448.4091	C-H bend	Alkane
1371.8204	0.728	511.0202	C-H bend	Alkane
1316.4043	0.706	550.5139	C-O stretch or C-N stretch	C-O bond or aromatic amine
1244.6087	0.707	52.8105	C-N stretch	Aliphatic amine
1152.2681	0.674	402.8943	C-O stretch	C-O bond
1021.1000	0.437	149.4168	C-H bend	Alkene
577.9747	0.507	18.1508	C-H bend	C-Cl bond
559.2007	0.021	12.4092	C-Br stretch	C-BR bond

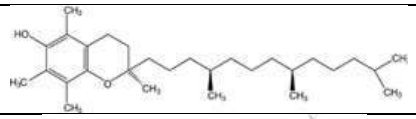
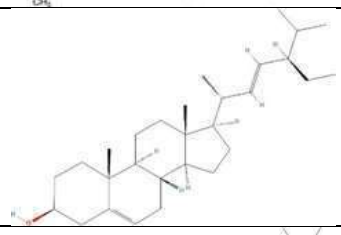
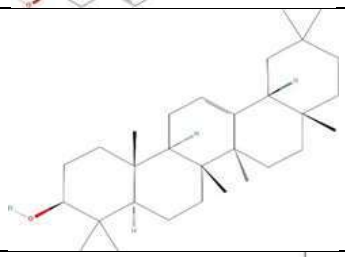
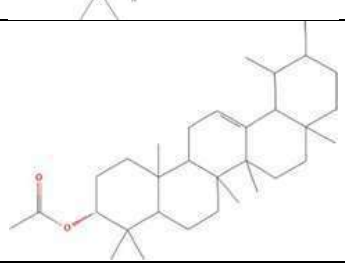
4. GC-MS (Gas Chromatography-Mass Spectroscopy)

Figure 9: GC-MS results for *Gymnema Sylvestre*

Table 11: Interpretation of Chromatogram[20-42]

Serial No.	Retention-n time	Area %	Structure	Compound	Qual
1	20.761	0.42		Varamol	96
2	23.033	1.03		1,2,3,4-Cyclohexanetetrol	92
3	26.918	40.26		Phosphonothioic acid, methyl-, S-(2-diethylaminoethyl), O-2-methylpropyl ester	50
4	28.362	1.123		Bicyclo[3.1.1]heptane, 2,6,6- trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)	60
5	28.493	0.40		-thiaundecane,2,10 diethyl-	53
6	29.625	0.20		lecanoic acid, 14-methyl, methyl ester	89
7	30.245	9.34		Palmitic acid	97
8	31.653	0.14		Margaric acid	94
9	32.234	1.40		Phytol	93

10	32.628	6.07		Oleic acid	98
11	32.902	2.06		Stearic acid	99
12	33.452	0.14		2h-indol-2-one, 1-(2,6- dichlorophenyl)-1,3-dihydro	99
13	35.134	0.12		4H-Dibenzo[de,g]quinoline, 5,6,6a,7-tetrahydro-1,2-dimethoxy-, (R)	49
14	37.415	0.54		4-hydroxyphenylbutazone	89
15	39.012	0.06		2-Ethylacridine	64
16	40.040	1.95		Erucamide	97
17	40.379	0.49		Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)	49
18	43.039	0.20		4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	53

19	43.504	1.72		Vitamin E	96
20	45.182	5.69		Stigmasterol	97
21	46.573	3.12		Beta.-Amyrin	94
22	47.915	2.07		Urs-12-en-3-ol, acetate	53

CONCLUSION

The pharmacognostic and phytochemical evaluation of *Gymnema Sylvestre* leaves validates its traditional use in herbal medicine. The presence of diverse bioactive compounds supports its potential role in managing diabetes and related disorders.

This study provides a scientific basis for further research and standardization of *Gymnema Sylvestre* as a medicinal plant-MS analysis revealed multiple bioactive compounds.

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