PHYTOCHEMICAL INVESTIGATION OF METHANOLIC EXTRACT OF *COCCINIA INDICA L*EAVES

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ABSTRACT

Diabetes or diabetes mellitus is part of a vast group of illnesses that originate from the body's ordinary function metabolic imbalance. The patient develops an enhanced blood glucose level above the normal range during the disease, and numerous other symptoms have been recognized, including increased urination frequency, enhanced thirst, increased hunger, and complications of vision. Mainly three diabetes mellitus (DM) kinds were recognized, including Type-1 DM (insulin-dependent), Type-2 DM (non-insulin-dependent) and Type-3 DM (gestational DM). With the tremendous growth of science, many allopathic drugs have been found over the previous centuries that have proved to be extremely effective in managing DM. Long-term use of these drugs, however, is hazardous to the general health situation of the patient and has created patient tolerance in many cases. For this reason, a lot of research has been carried out in different crops to discover effective herbal products that can effectively assist in managing DM. Herbal based drugs are much safer with fewer side effects and, unlike allopathic drugs, they can be used for a long time with almost no hazardous side effects. The purpose of the present review is to provide an overview of DM, its different kinds, symptoms, and multiple herbal products presently being efficiently used to manage DM.

Keywords: *Diabetes mellitus, metabolic balance, blood glucose level, allopathic medicine, Herbal medicine.*

INTRODUCTION

DM belongs to the group of heterogeneous metabolic disease which is characterized by increased blood glucose levels.(Ubaid *et al.*, 2019) DM is characterized either by decrease levels of insulin production from the pancreas or by body resistance towards insulin which leads to alteration of glucose levels in blood.(Deshmukh and Jain, 2015) In the past century, DM has been simply classified into two groups. The first group is type-1 DM and the second group is type-2 DM. type-1 accounts for about 5-10% of all diabetes cases, while type-2 accounts for about 90-95% of cases.(Karalliedde and Gnudi, 2016) In current days, various other types of DM have been classified and these include type-3 (gestational DM) and type-4 which is related to patient genetic predisposition, or DM induced by other agents including drugs.(Goldenberg and Punthakee, 2018) Previously the type-1 DM was known as insulin dependent caused by autoimmune damage of pancreatic β-cells varies from patient to patient. The type-1 DM represents the most severe type of DM and patients with it required timely infusion of insulin.(American Diabetes Association., 2004) The type-2 DM was previously

called as non-insulin dependent. The causes of type-2 DM are appointed to the life style, genetic predisposition, and environmental factors. It is characterized by decreased insulin production, insulin resistance by the body and hyperglycemia.(Baynest, 2015) The type-3 (gestational DM) takes place during the pregnancy and it is mainly characterized by irregular metabolism of the carbohydrate by the pregnant woman which leads to increased levels of blood glucose. This type of DM affects nearly 7% of the total population of pregnant women.(Ubaid *et al.*, 2019) The other types of DM are the result of various causes, genetic predisposition being the most significant cause.(Goldenberg and Punthakee, 2018) A holistic approach must be adopted during the education of the population of patients and healthy people about DM because the disease not only affects the patient health it has also other implications such as at family level, social and psychological level.(Kosti *et al.*, 2012)

1.1 Authentication of Drug

All experiments were performed in the Pharmacognosy research laboratory of the Pharmacy department and a herbarium specimen (HIMT/Pharm/Herb/2018/1802) was also deposited.

S. No.	**Vernacular Names**
1.	Hindi**:**Parval, Tindora, Tinda, Kundru;
2.	Marathi**:** Tindora, Tondli;
3.	English**:**Scarlet;
4.	Spanish**:**Pepino, cimaron;
5.	Danish**:**Skariagenagurk;
6.	Japanese**:**Yasai, karasuuri;
7.	Malay**:**Pepasan, Kovakka, Kovai;

Description Perennial; tuberous roots; smooth stem; white flowers; pentagonal or triangular leaves; sub-flashy; (**Mayank and Shashi, 2013)** flowers:-star shaped; axillary clusters; 3 stamens; **(Gupta *et al*., 2008)**. fruits: -glabrous, pulpy, ovoid; ellipsoid shaped; **(Gupta *et al.,* 2008)**	

1.2 PHYTO-CHEMICAL SCREENING

The concentrates of root was subjected to different subjective substance tests to decide the nearness of different phytoconstituents like alkaloids, glycosides, sugars, phenolic etc. Utilizing detailed techniques in (Khandelwal et al (2007).

Alkaloids: The weak HCL corrosive was combined with the dried concentrate, mixed firmly, and then separated. The unique test was also conducted using the filtrate.

- Mayer's test: Mayer's reagent was used to prepare the filtrates (potassium mercuric iodide). The placement of a yellow cream hasten shows how close alkaloids are..
- Wagner's test: Wagner's reagent was used to treat the filtrates . Alkaloids are present because no brown and reddish-brown precipitate occur.
- Dragendorff's test: Dragendorff's reagent was used to prepare the filtrates. Red hasten's arrangement shows how close the alkaloids are..
- Hager's test: Hager's reagent was used to treat the filtrates. The placement of yellow hasten shows how close alkaloids are..

Carbohydrates

- Molisch's test: Concentrated sulphuric corrosive was added precisely at the test tube's edges after extracts were treated with a dipsomaniac setup in the test tube. Starches are close by as evidenced by the growth of the violet ring at the crossing.
- Fehling's test: The filtrate was heated for 5–10 seconds over a water shower after adding the Fehling A and B arrangement. Next, it was killed with soluble base and fermented with moderate hydrochloric acid. Initially yellow, a block red acceleration then signaled the imminence of decreasing sugars.

Glycosides

Selection of concentrate's residual sugar content, and then hydrolyze the extract with a mineral corrosive. Decide once more what the hydrolyzed remove's total sugar content is. Glycosides are revealed by an increase in sugar concentration..

- Legal's test: The concentration was treated using $C_5H_4FeN_6Na_2O_3$ in pyridine and methanolic base. The pattern of pink and red colors indicated the proximity of cardiac glycosides.
- Killer Killani test: A small amount of dry concentrate was dissolved in two milliliters of icy acidic solution that contained one drop of ferric chloride solution. After the expansion of 1 ml of concentrated H2SO4, these remained stable for quite some time. A darker ring acquires at the nearness of cardenolides.

Saponin glycosides

- Froth test: The focus was weakened by shaking for 15 minutes in a graded barrel with 20ml of refined water on its own. Saponins can be detected by a layer of foam that is about 1 centimeter thick.

Flavonoids

- Shinoda test: Since being purged with 10ml of ethanol for 15 minutes in a water shower, specific dry concentrations were filtered .The filtrates were mixed with a tiny amount of magnesium bind and concentrated HCl. The increase of pink shading demonstrates how related flavonoids are.

- FeCl3 test: FeCl3 arrangement is placed in the sample arrangement. The change in shade from green to dark indicates the presence of flavonoids..

Amino acids & Protein

- Million's test: Million's reagent was used to handle the concentrations. The configuration of a white particle, which turned red upon warming, demonstrates the proximity of proteins and amino acids.
- Ninhydrin test: 0.25 percent Ninhydrin reagent was added to the concentrates and bubbled for a short while. The placement of blue shading indicates the proximity of amino acids.

Tannins

- FeCl3 test: 2-3 drops of neutral $FeCl_3$ were taken to the concentration as a treatment. a remedy (5 percent). The arrangement of dark shades of pale blue indicates the presence of tannins.
- Lead acetic acid derivation test: The 10% concentration was treated with a few drops of neutral Lead acetic acid synthesis. The quickness with which yellow appears is evidence of tannins.

Triterpenoids and Steroids

- Liebermann Burchard response; A mixture of ethanol, ethanol-chloroform-ethyl acetic acid derivation, and ethanol will be applied to the dried amounts. To the aforementioned mixture, 1 to 2 drops of a strong sulfuric corrosive must be added. The presence of steroids is indicated by the Solution's subtle green tint, whilst triterpenoids are indicated by the arrangement's faint pink color.
- Salkowaski response: After a few drops of concentrated sulfuric acid corrosive were added, chloroform was added to the dry concentrations, thoroughly agitated, and permitted to sit for a while. The presence of triterpenoids was indicated by the appearance of a yellow tint in the lower layer, whereas the presence of steroids was indicated by the appearance of red shading in the lower layer.

1.3 Determination of moisture (loss on drying)

Firstly, 3gram of shade-dried drug will be taking into a weighed flat and thin porcelain dish. Then dried in the oven at 100° or 105° after that cool in desiccators and calculate the loss in weight is usually recorded as moisture (Khandelwal 2006).

Loss on drying (%) = (Weight loss/Weight of drug) X 100

1.4 Determination of Foreign Matter

250 g of leaves will take and Spread it in a thin layer and sort the foreign matter into groups either by visual inspection. Weigh the portions of this sorted foreign matter to within 0.05 g. This method is followed from W.H.O. (2011).

Calculate the content of foreign matter

% foreign matter = Weight of shorted foreign matter/Weight of drug) × 100

1.5 DETERMINATION OF ASH VALUE

1.5.1 Total ash

Firstly weigh and ignite flat, thin porcelain dish silica crucible and then weigh about 2g of powdered drug into the dish/ crucible and incinerated in a crucible at a temperature 500-

600˚C in a muffle furnace till carbon free ash will obtained then it is cool, weight and percentage of yield will calculated as per reference (Khandelwal 2006) and (WHO guidelines 2002).

Total ash (%) = (weight of ash/ weight of drug) x 10

1.5.2 Determination of acid-insoluble value

After as per the above step ash using 25 ml of dilute-hydrochloric acid wash the ash from the dish using for total ash into a 100ml beaker. Then place wire gauze over a Bunsen burner and boil for five minutes. Filter through an ash-less filter paper, wash residue twice with hot water. Ignite the crucible in the flame cool and weigh, after that put the filter paper and residue together into crucible; heat gently until vapors cease to be evolved and then more strongly until all carbon will remove. Cool in a dedicator weigh the residue and finally calculate acid insoluble ash of the crude drug with reference to the air dried sample of the crude drug. (Khandelwal 2006)

Acid insoluble ash (%) = (weight of acid insoluble ash/weight of drug) x 100

1.5.3 Determination of water soluble ash

Total ash will boiled for 5 min with 25 ml water and insoluble matter which will collect on an ash-less filter paper will washed with hot water and ignited for 15min at a temperature not exceeding 450˚C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash will calculated with reference to the air-dried powered drug**.**

Water soluble ash (%) = (Weight of water soluble ash/Weight of drug) × 100

1.5.4 Determination of Sulfated Ash

A silica or platinum crucible will heated to redness for 10 minutes, allowed to cool in desiccators and weigh. Unless otherwise specified in the individual monograph, 1g of the substance will transfer to the crucible under examination and the crucible and the content will weigh accurately. Gently, ignite at first until the substance will thoroughly charred. The residue will be cool and moistened with 1ml of sulphuric acid, gently heated until the white fumes will be no longer evolved and ignited at 800̊ ± 25̊ until all black particles will disappear. The ignition will be conducted in a place protected from air currents. The crucible will be allowed to cool and few drops of sulphuric acid will add and heated. Ignite again as before, allowed to cool and weigh. The operation will repeat until two successive weighing do not differ by more than 0.5mg (Indian Pharmacopoeia 2010)

Sulphated ash (%) = (weight of sulphated ash/ weight of drug) x100

1.6 Quantitative Evaluation of Phyto-Constituents

1.6.1 Determination of Total Phenolic Content

The total phenolic content of all medication concentrates was evaluated using the procedure outlined in the Ayurvedic Pharmacopeia of India. The total phenolic content per gram of concentrate is represented as a reciprocal of gallic corrosive milligrams. A methanol (1 mg/ml) stock of the concentrate will be prepared. The 25 ml volumetric flagon will be filled with 10 ml of water, 1.5 ml of the folin ciocalteau reagent, and the correct amount of concentrate from the stock arrangement. A 20 percent sodium carbonate solution and up to 25 milliliters of twice-refined water were added after the first five minutes. The blend's absorbance at 765 nm was assessed after 30 minutes. The amount of total phenolics was calculated from the adjustment bend of gallic acid using the aforementioned method and reported in conformity with the Ayurvedic Pharmacopeia of India (2008).

Reagents

Sodium carbonate reagent (20%): After being broken up into 20 g of sodium carbonate and 100 ml of water, it was separated after being given a medium-term Gallic corrosive stock solution (1 mg/ml): Disintegration of 10 mg of gallic corrosive in 10 ml of purified water.

1.6.2 Determination of Flavonoids Content

For calculating the total amount of flavonoids in the concentrated plant root, the aluminum chloride method was used. Concentrate arrangements have been taken in aliquots, each of which contains 3 ml of ethanol. 2.8 ml of pure water, 0.1 ml of a potassium acetic acid derivative , and 0.1 ml of aluminum chloride (10%) were then gradually added. The test setup was continuously shaken. After brooding for 30 minutes, absorbance at 415 nm is measured.. A std. adjustment plot created at 415 nm utilizing known groupings of rutin According to Mervat and Hanan, the convergences of flavonoids in the test samples were calculated from the alignment plot and reported as mg rutin identical/g of the example. (2009).

Reagents

1. Aluminum chloride arrangement 10 %: 100 ml of methanol was used to dissolve 10g of aluminum chloride.
2. 1M Potassium acetic acid derivation arrangement: 9.815g $C_2H_3KO_2$ derivation was broken up in 100 ml of refined water.

Standard rutin arrangement (1mg/ml): 10 mg rutin was broken up in 10 ml methanol.

2.0 RESULTS

Phytochemical screening Qualitative analysis of *Coccinia indica* leaves showed the presence of SPMs like Flavanoids; Phenolic Compounds; Terpenoids; Reducing sugars; alkaloids; glycosides; steroids; Amino acids; Proteins; phytosterols and carbohydrate etc.

Presence / absence of PPMs and SPMs in *Coccinia indica* leaves

Tests performed for	Leaves
Alkaloid	+
Glycosides	+
Terpenoids	-
Steroids	+
Proteins & Amino acids	+
Saponins	-
Phytosterols	-
Flavanoids	+
Phenolic Compounds	+
Tannins	+
Fats	-
Reducing sugars	+
Carbohydrate	+

Table 2.1 : Physicochemical parameters of leaves of *Coccinia indica.*

Quantitative parameter	Values obtained (%) w/w
Alcohol Soluble Extractive Value (ASEV)	8.6 %
Water Soluble Extractive Value (WSEV)	24.80 %
Total Ash Value (TAV)	15.53 %
Acid Insoluble Ash Value (AIAV)	1.783%
Water Soluble Ash Value (WSAV)	8.93%
Swelling Index (SI)	1.9%
Loss on Drying (LOD)	10.32%
Foreign Matter Content (FMC)	1.4%

2.2 DETERMINATION OF TOTAL PHENOLIC CONTENT

Gallic acid's standard curve served as the basis for the quantitative determination of total phenols, and linearity of the calibration curve was attained (figure 6.3). The total phenolic content of the Methanolic extracts of *Cocina India*.

Table 2.2 Total phenolic content of Gallic acid (standard drug)

CONCENTRATION (ug/ ml)	ABSORBANCE ƛ max = 765 nm
0	0.000
2	0.107
4	0.234
6	0.362
8	0.511
10	0.621
12	0.822

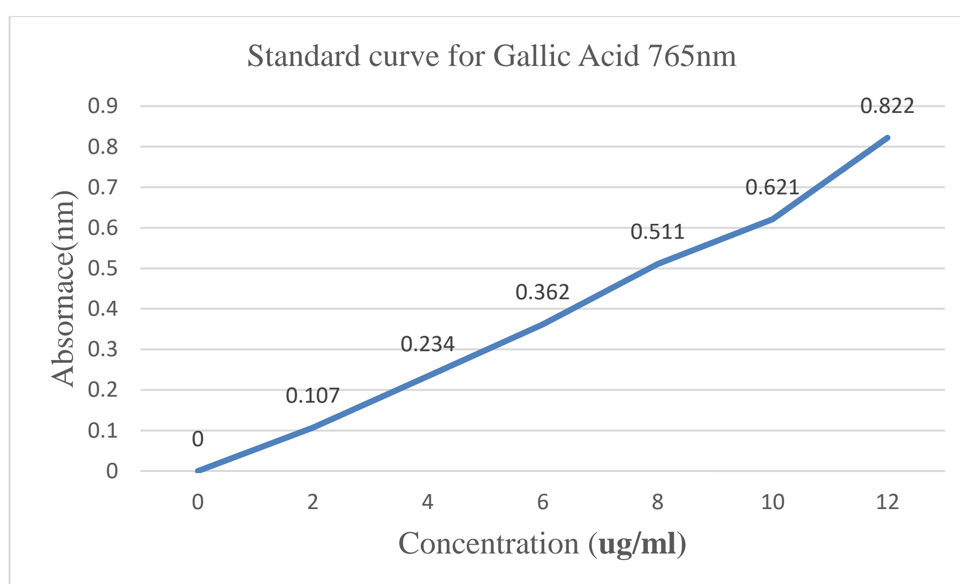
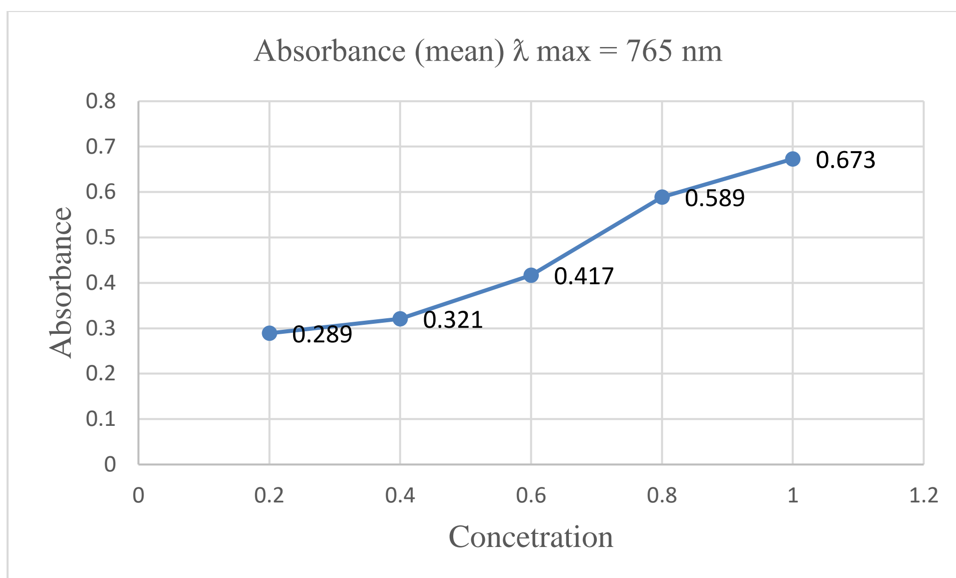


Fig. 2.1 Calibration Curve of Standard Curve of Gallic Acid (y = 0.134 x - 0.159 R^2 = 0.993

Table 2.3 Absorbance of *cocina indica* (Methanolic Extract)

Concentration (ml)	Absorbance (mean) ƛ max = 765 nm
0.2	0.289
0.4	0.321
0.6	0.417
0.8	0.589
1.0	0.673



2.3 DETERMINATION OF TOTAL FLAVONOIDS CONTENT

The quantitative estimation of the total flavonoids content was built on Rutin's standard curve, and linearity of the calibration curve was reached (figure 6.5). It was also examined how much flavonoids were present overall in the hydroalcoholic extracts of Zizyphus xylopyrus and Embelia ribes. The quantity of flavonoids in the hydroalcoholic extract is shown in Table 6.8..

Table 2.4 Total Flavonoids Content of Rutin

CONCENTRATION (ug/ ml)	ABSORBANCE
0	0
10	.132
20	.234
40	.424
60	.602
80	.707
100	.989

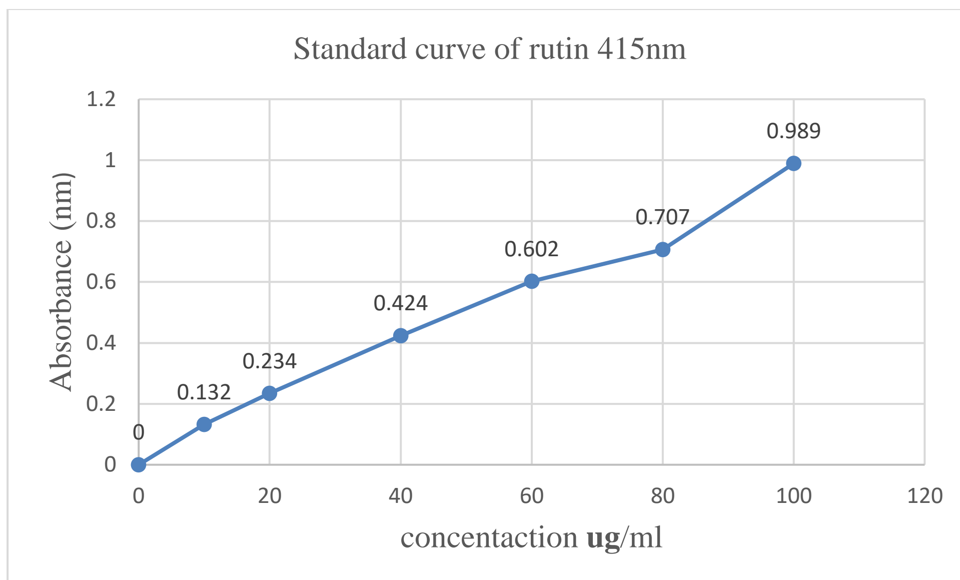


Fig.2.3 Calibration Curve of Standard Curve of Std. Curve of Rutin ($y = 0.009x + 0.029$ $R^2 = 0.99$)

Table 2.4 Absorbance of *cocina indica* (Methanolic extract)

Concentration (µg/ml)	Absorbance (mean) ƛ max = 415 nm
0.2	0.020
0.4	0.111
0.6	0.231
0.8	0387
1.0	0.477

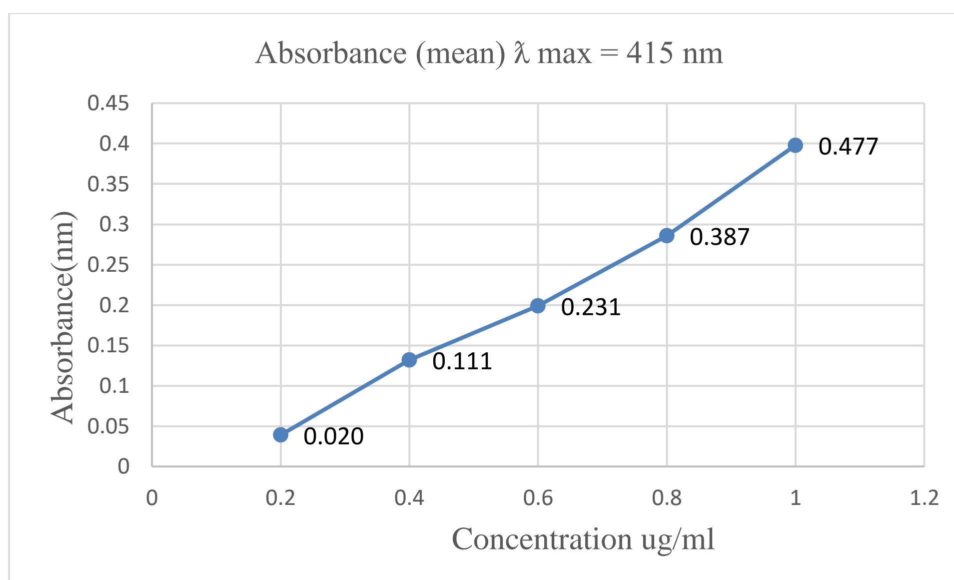


Fig. 2.6 Methanolic extract test chemical absorbance ($y = 0.436x - 0.050$ $R2 = 0.993$)

High amount of total phenolics content (1286.16 ± 6.78 mg GAE/100 gDW) and total flavonoids contents (9.84 equivalents/100 mg) were found in the MECIL Extract. MECIL-C extract have showed glucose lowering effect after 2 hr of treatment and sugar levels were restored after 3 hr. MECIL extract C had alleviated glucose level. MECIL-C produced better action than MECIL-B and MECIL-A extracts.
Further, MECIL-C, MECIL-B and Quercetin have prominent hypolipidemic / hypocholesterolemic activity but lesser than Standard drug. MECIL-C extract treatment alleviated the level serum cholesterol. MECIL-C extract treatment restored HDL level. MECIL-C extract alleviated VLDL level. Finally, much more elaborated phytochemical pharmacological research is recommended.

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