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DEVELOPMENT AND EVALUATION OF *TERMINALIA CHEBULA* HERBAL MOUTHWASH

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Abstract

This study investigates the antibacterial potential of *Terminalia chebula* against *Streptococcus mutans*, a primary cause of dental caries. An herbal mouthwash was formulated using aqueous extracts of *T. chebula*, honey, and polyethylene glycol. Phytochemical screening revealed the presence of bioactive compounds like tannins, flavonoids, and saponins. GC-MS analysis identified a complex chemical profile dominated by antioxidant-rich compounds, including siloxanes, phosphites, and natural esters. The formulation exhibited significant antibacterial activity, with Formulation F3 showing 98.88% microbial reduction. The mouthwash demonstrated physical stability over 30 days, maintaining consistent color, odor, taste, and pH. The findings support the use of *T. chebula* in dental hygiene products as a natural alternative to synthetic oral care agents, highlighting its potential in preventing plaques, caries, and gum-related diseases. Regular use of *T. chebula* mouthwash led to good oral health and long-term oral well-being.

INTRODUCTION:

Mouthwash is a liquid formulation designed to cleanse the mouth and teeth, refresh breath, and manage plaque along with other dental issues. [1,2] The mouthwash circulated through the muscles surrounding the mouth to eliminate oral bacteria. [3] There are two types: chemical and herbal. Herbal mouthwashes have natural ingredients with

anti-inflammatory and antimicrobial properties, while chemical mouthwashes contain ingredients like chlorhexidine and cetylpyridinium chloride that can whiten and reduce microbial load but may have side effects like tooth discoloration, altered taste, and gum irritation. [4,5] Mouthwash helps reduce germs, prevent plaque buildup, and maintains oral hygiene, often used as an adjunct to brush and flossing. Herbal mouthwashes are suitable for longer use to control dental problems, whereas chemical mouthwashes are recommended for short-term use. [6]

Terminalia chebula, also known as Haritaki, is a plant with therapeutic properties and is highly valued in Ayurvedic medicine. Its fruits contain compounds that provide antimicrobial, astringent, antioxidant, and anti-inflammatory benefits. [7,8,9] Studies show that *T. chebula* extract fights oral pathogens effectively. [10] GC-MS analysis, a powerful tool used to identify and quantify the bioactive compounds in plant extracts, has revealed a complex chemical profile of *T. chebula*, rich in bioactive compounds. The GC-MS analysis of *T. chebula* extract has identified several bioactive compounds, which contribute to its therapeutic potential. Making a mouthwash with *T. chebula* is a promising natural alternative, especially for those preferring natural health products. *Terminalia chebula* mouthwash effectively helps prevent and treat tooth decay, promoting overall oral health. [11]
Regular use can lead to good oral health, crucial for maintaining long-term oral well-being and preventing future dental issues. The benefits of using *Terminalia chebula* mouthwash make it an attractive option for those seeking a natural and effective method to oral hygiene. [12,13]

MATERIAL AND METHODS:

Collection of Drugs:

In the month of February, *Terminalia Chebula* fruit was collected from plants in College Herbal Garden, Shivajirao S. Jondhle College of Pharmacy, Asangaon.

Preparation for Extraction:

The dried fruits of *Terminalia chebula* were first washed thoroughly with distilled water to remove any surface impurities and then dried at room temperature (25–30°C) for 7–10 days to eliminate moisture. Once completely dried, the fruits were coarsely broken and pulverized using a stainless-steel grinder. The resulting powder was passed through a 60-mesh sieve to obtain a uniform fine powder. Take *Terminalia chebula* powder and mix with 100 ml of distilled water in a beaker, to get a uniform suspension. The mixture is then subjected to cold maceration at 4°C for 72 hours. After maceration, the suspension is filtered through muslin cloth to remove coarse particles, resulting in a clarified filtration. The filtrate is then concentrated at 40°C using a heating mantle to remove excess solvent. The concentrated extract is dried to obtain a solid residue (dried extract). [6,14]

Phytochemical Screening:

1)Test for Alkaloids:
Dragendroff's test:
Dissolve different extracts of the herbal medicine in chloroform. Remove the chloroform through evaporation and then acidify the remaining substance by adding a few drops of Dragendorff's reagent (Potassium Bismuth Iodide). The appearance of a reddish-orange precipitate signifies the presence of alkaloids.
2) Test for Saponins:
Foam test:
A small amount of extract was taken in a test tube with a small amount of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins.

3) Test for Phenolic compounds:
Ferric chloride test:
Measure 2 ml of the extract and transfer it to a test tube, then slowly add ferric chloride solution drop by drop. The appearance of a bluish-black precipitate indicates the presence of phenolic substances.

4) Test for Tannins:
Lead Acetate test:
Measure 2 ml of the extract and transfer it to a test tube, then slowly add lead acetate solution drop by drop. Appearance of White precipitate indicates presence of Tannins.

5) Test for Proteins:
Ninhydrin test:
Add a few drops of Ninhydrin into the extract Appearance of blue colour indicates presence of amino acid whereas proteins may rarely give positive results.

6) Test for Carbohydrates:

Molisch's test:
Mix the extract with Molisch's reagent and gently introduce concentrated H2SO4 along the sides of the test tube to form distinct layers. The presence of carbohydrates is indicated by the emergence of a red violet ring at the edge.

7) Test for Flavonoids:
Ferric chloride test:
Add a few drops of neutral ferric chloride solution to the extract. The emergence of green color indicates the existence of flavonoids.

8) Test for Glycoside:
Legal's test:
To aqueous extract, add 1ml Pyridine and 1ml sodium nitroprusside. The existence of glycosides is reflected by a color shift from pink to red.

Formulation of Mouthwash:

9) Test for Terpenes:
Salkowski Test:
Add 2 ml of chloroform to 5 ml of extract. Add 3 ml of concentrated sulfuric acid slowly, allowing it to form a separate layer. Observe for a reddish-brown coloration at the interface.

10) Test for Fixed Oil:
Spot Test:
Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oils. [15,16,17]

Phytochemical analysis:

The sample was dissolved in Methanol and injected in a GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Split less. The conditions for operating the GC–MS during the analysis were established as follows: The oven was first preheated to 45 °C for 2 minutes, after which the temperature was increased to 140 °C at a rate of 5 °C per minute. Eventually, it was raised to 280 °C and maintained at that level for 10 minutes. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out at 70 eV. The GC run time was between 9.11 minutes and 52.0 minutes. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L) [18,19,20]

Table .1: Formulation of Mouthwash

Ingredients	Function	F 1	F 2	F 3
T.Chebula extract	Active Drug	2.5 gm	5 gm	10 gm
Polyethylene glycol 400 (20 % v/v)	Solubilizer	20 ml	20 ml	20 ml
Honey	Sweetener	6 ml	6 ml	6 ml
Peppermint oil	Flavorings Agent	2-3 Drops	2-3 Drops	2-3 Drops
Distilled Water	Vehicle	Q.S to 100 ml	Q.S to 100 ml	Q.S to 100 ml

A 20% v/v solution of Polyethylene Glycol (PEG 400) was prepared by mixing 20 ml of PEG with 80 ml of distilled water and stirring the mixture until a uniform solution was obtained. Subsequently, dried *T. chebula* extract was weighed and suspended in the prepared PEG solution. The suspension was stirred using a stirrer for 10 to 15 minutes to ensure thorough dispersion of the extract within the solvent. The resulting mixture was then placed into a heating mantle. Heating continued until a semi-solid or concentrated residue was obtained. Honey was added to the concentrated extract, and the mixture was stirred thoroughly to form a uniform concentrate. The concentrate was diluted with distilled water to obtain 100 ml of mouthwash. [6,14]

Evaluation parameters:

1. **Physical Examination**: Through visible examination, physical characteristics including odour and colouring have been checked [21,22].
2. **pH**: A virtual pH meter was used to measure the pH of a prepared natural mouthwash. The pH meter was calibrated with the aid of a modern buffer A pH meter was used to assess the mouthwash's pH after it had been weighed, dissolved in 50 cc of pure water, and weighted again [21].
3. **Temperature**: During this test, mouthwash is stored either at room temperature, 25^0C, or in the refrigerator, 8 degrees Celsius [23] [24]

4. **Viscosity**: With an Ostwald viscometer, the mouthwash's viscosity was determined.

5. Screening for Antimicrobial Potential: [25,26]

Name of Test:
ASTM E 2315-2023
Assessment of antimicrobial activity using a Time-kill procedure
Test Organism:
Streptococcus Mutans ATCC 25175

Test Procedure:
The product was inoculated with test organisms individually (approximately 10^6CFU/ml). After the specified exposure time, the surviving microorganisms recovered by drawing an aliquot, neutralizing it and performing the standard pour plate technique. Culture count was ascertained by dilution blank. Adequate validation of

neutralizing agents was also carried out. Tests were carried out in duplicate and the average count was taken CFU/ml.

Experimental conditions:
Test Product: 10% conc.
Neutralizer: Tween 80-100 ml, Lecithin 30g, Sodium Thiosulphate 5.0g, L histidine 1.0g, Phosphate buffer solution of 100 ml in 1L.
Contact Time: 5 minutes
Contact Temperature: 20ºC ± 1ºC
Growth Media: Soyabean-casein digest agar at 37ºC for 48 hours
Incubation Condition: 37ºC for 48 hours
6.Centrifugation test: Using test tubes, the prepared mouthwash was centrifuged in the centrifugation machine to examine the phase separation [27] [28].
7. Microscopic test: A compound microscope with magnification powers of 10 and 40 was used to assess the mouthwash formulation's transparency [21]

8.Procedure for Physical Stability Testing of Mouthwash: [29]

The physical stability of Three formulations (F1, F2, F3) was assessed at three intervals: Day 1, Day 15, and Day 30. The following parameters were evaluated:
1) Colour : A 5 ml sample was transferred to a clear glass vial and observed against a white background under daylight.
2) Odour : Vial uncapped, odour checked by gently wafting the aroma toward the nose.
3) Taste : Small samples tasted cautiously palate cleanse before and after.
4) pH : Calibrated digital pH meter used; electrode immersed in the sample to record pH.
5) Sedimentation : Sample stored undisturbed in a glass vial for 24 hours and observed for any sediment.
6) Centrifugation : Using test tubes, the prepared mouthwash was centrifuged in the centrifugation machine to examine the phase separation
7) Temperature : During this test, mouthwash is stored either at room temperature, 25 ^{0} C, or in the refrigerator, 8 ^{0} Celsius
8) Viscosity : With an Ostwald viscometer, the mouthwash's viscosity was determined.

RESULT:

Table .2 : Phytochemical analysis of extract

Sr. no	Test	Observation	Inference
1.	Alkaloids	Yellow precipitate is observed	Alkaloids is Absent
2.	Saponins	Foam was formed	Saponin is Present
3.	Phenolic compounds	Bluish black precipitate was observed	Phenolic compounds Present
4.	Tannins	White precipitate was observed	Tannins is Present
5.	Proteins	Yellow colour was observed	Proteins is Absent
6.	Carbohydrates	Yellowish orange colour was observed	Carbohydrates is Absent
7.	Flavonoids	Green colour was observed	Flavonoids is Present
8.	Glycoside	White precipitate was observed	Glycoside is Absent

9.	Terpenes	Yellowish orange colour was observed	Terpenes is Absent
10.	Fixed oil	Oil staining was absent	Fixed oil is Absent

Phytochemical Analysis:

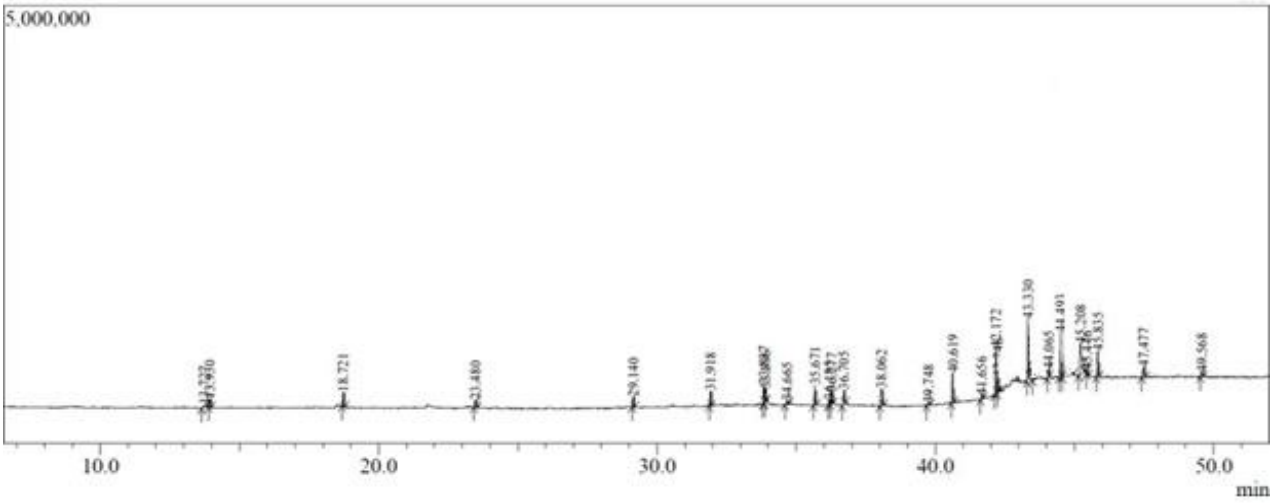

Figure .2 : GC-MS chromatogram of aqueous extract of *Terminalia chebula* dried fruit.

Table .3: GS-MS identification of compounds

Peak	R. Time	Area	Area %	Height	Name	Common Name
1	13.725	430563	1.00	34901	Cyclopentasiloxane , decamethyl-	Decamethylcyclopentasiloxane (D5)
3	18.720	366305	0.82	167119	Cyclohexasiloxane, dodecamethyl-	Dodecamethylcyclohexasiloxane (D6)
4	23.48	273086	0.62	87153	Cycloheptasiloxane , tetradecamethyl-	Tetradecamethylcycloheptasiloxa ne
5	29.12	836219	1.93	121268	Cyclooctasiloxane, hexadecamethyl-	Hexadecamethylcyclooct asiloxane (D8)
6	31.917	2531875	5.91	180215	Cyclononasiloxane, octadecamethyl-	Octadecamethylcyclonona Siloxane
7	33.836	357072	0.86	222868	Cyclodecasiloxane, eicosamethyl-	Eicosamethylcyclodecasil Oxane
8	33.886	325784	0.79	162121	Hexadecanoic acid, methyl ester	Methyl palmitate
11	36.165	132652	0.34	57223	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	Methyl linoleate

12	36.267	396658	0.89	149431	9-Octadecenoic acid, methyl ester, (E)-	Methyl oleate
13	36.805	523845	1.83	177805	Methyl stearate	Methyl octadecenoate
17	42.272	4135747	9.56	564114	Tetracosamethyl-cyclododecasiloxane	Tetracosamethylcyclododecasiloxane
21	44.764	29154278	65.25	3307323	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Tris (2,4-di-tert-butylphenyl) phosphite
22	45.218	1824177	4.35	385436	13-Docosenamide, (Z)-	Erucamide
23	45.644	131234	0.27	53074	Squalene	Squalene

Evaluation of Mouthwash:

1. Physical Examination: visually examining the physical characterization of the medicament was performed, and the results are given in the table for various physical characterizations

Table 4: Physical examination of prepared mouthwash

Formulation	Color	Odor	Taste	Appearance
F1	Reddish Brown	Characteristic	Slightly Bitter	Clear
F2	Reddish Brown	Characteristic	Slightly Bitter	Clear
F3	Reddish Brown	Characteristic	Slightly Bitter	Clear

2. pH observation: To measure pH, we used pH paper. A piece of pH paper was dipped into 5 ml of mouthwash. It supported a shadeation that identified the pH range between 6 to 7 by comparing it to a recognised pH shadeation range. Thus, the pH that was found between 6 to 7 [1]

Table 5: Results of pH examination of prepared mouthwash

Formulation	pH
F1	5.95
F2	6.15
F3	6.38

3 .Microbial Test: The mouthwash was inoculated on agar medium plates using the streak plate method, and a control was created. The plates were placed in the incubator and let to incubate for 24 hours at 37°C. After the incubation period, the plates were removed and compared to the control to see if any microbial growth had occurred. When they were inoculated in the agar medium, they did not produce any microbial growth, indicating that the formulation was free of microorganisms.

Table 6: Antimicrobial Testing

Sample Description	Test Organism	Exposure Time	Microbial Count		Antimicrobial Log Reduction	Antimicrobial Percentage Reduction
			Count	Log		
F 1	Streptococcus Mutans ATCC 25175	Initial	1.9×10^4	1.21	-	-
		5 Minutes	2.20×10^2	0.725	0.5	25.82
F 2	Streptococcus Mutans ATCC 25175	Initial	3.69×10^4	2.43	-	-
		5 Minutes	4.60×10^2	1.45	0.99	53.44
F 3	Streptococcus Mutans ATCC 25175	Initial	7.20×10^4	4.85	-	-
		5 Minutes	8.00×10^2	2.90	1.95	98.88

4. Temperature: Following exposure to various storage temperatures, the physical features of various mouthwash formulations.

Table 7: Mouthwash formulation exposure to different temperature

Formulation	Room temperature	Refrigerator temperature	Time period	Changes
F1	25° C	2-8 °C	1 week	No change
F2	25° C	2-8 °C	1 week	No change
F3	25° C	2-8 °C	1 week	No change

5.Microscopic test: -by using compound microscope with magnification powers of 10 and 40, it was observed that the mouthwash formulations are transparent was used to assess the mouthwash formulation's transparency.

Table 8: Result of the microscopic test of mouthwash formulations

Formulation	Microscope	Magnification power	Observations
F1	Compound microscope	10&40	Transparent
F2	Compound microscope	10&40	Transparent
F3	Compound microscope	10&40	Transparent

6.Centrifugation test: -Using centrifuge tubes, the prepared mouthwash was centrifuged in the centrifugation machine to examine the phase separation and it was found that there is no phase separation.

Table 9: Result of centrifugation test of mouthwash formulations

Formulation	Speed (rpm)	Time (min)	Appearance before test	Appearance before test	Stable/Unstable
F1	3000-5000	15-30	clear	No phase separation	Stable
F2	3000-5000	15-30	clear	No phase separation	Stable
F3	3000-5000	15-30	clear	No phase separation	Stable

Table. 10: Physical Stability of Mouthwash

Parameters	**F1**			**F2**			**F3**		
	Day 1	Day15	Day30	Day1	Day15	Day30	Day1	Day15	Day30
Colour	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown	Reddish Brown
Odour	Characteristic	No Change	No Change	Characteristic	No Change	No Change	Characteristic	No Change	No Change
Taste	Slightly Bitter	No Change	No Change	Slightly Bitter	No Change	No Change	Slightly Bitter	No Change	No Change
pH	5.95	5.95	5.95	6.15	6.15	6.15	6.38	6.38	6.38
Sedimentation	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Centrifugation	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable
Temperature	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Viscosity	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change	No Change

DISSCUSION:

The present study aimed to evaluate the phytochemical profile, antimicrobial efficacy, and physical stability of the formulated *Terminalia chebula* mouthwash. Aqueous extracts of *Terminalia chebula* were subjected to preliminary phytochemical screening, GC-MS analysis, antimicrobial testing, and physical stability assessment across different formulations (F1, F2, and F3).

Screening for phytochemicals showed the presence of multiple bioactive components. Notably, saponins, phenolic compounds, tannins, and flavonoids were found to be present, which are well-documented for their antioxidant and antimicrobial activities. The absence of alkaloids, proteins, carbohydrates, glycosides, terpenes, and fixed oils suggests a more selective extraction of polyphenolic compounds in the aqueous medium. The strong presence of saponins and flavonoids supports the traditional use of *Terminalia chebula* in oral care due to their ability to inhibit bacterial adhesion and biofilm formation.

GC-MS analysis of the aqueous extract identified a range of chemical constituents, with their relative concentrations expressed as Area % (refer to Figure 2 and Table 3). Tris(2,4-di-tert-butylphenyl) phosphite was the most abundant compound identified, making up 65.25% of the total composition. This compound, a phenolic phosphite antioxidant commonly utilized in industrial processes, suggests potential antioxidant capabilities within the extract. The second most abundant component was Tetracosamethylcyclododecasiloxane (9.56%), a silicone-based substance frequently incorporated into cosmetic formulations. Other notable compounds included Octadecamethylcyclononasiloxane (5.91%) and Erucamide (4.35%), the latter being a fatty acid amide recognized for its lubricating and anti-static functions. Minor quantities of other siloxanes, such as Decamethylcyclopentasiloxane (D5) at 1% and Hexadecamethylcyclooctasiloxane (D8) at 1.93%, were also observed. Additionally, the extract contained fatty acid esters like Methyl palmitate (0.79%) and Methyl linoleate (0.34%), both of which are associated with skin-conditioning and antioxidant benefits. A small amount of Squalene (0.27%)—a natural compound with antioxidant and skin-protective properties—was also present. Collectively, these findings highlight the extract's composition as rich in bioactive compounds, particularly siloxanes, phosphites, and natural esters, which may contribute to its antioxidant potential.

Antimicrobial testing against *Streptococcus mutans,* a key etiological agent in dental caries, showed variable but promising results across the three formulations. Formulation F3 demonstrated the highest antimicrobial efficacy, with a 98.88% microbial reduction and a log reduction of 1.95 after 5 minutes of exposure. Formulation F2 showed moderate activity with a 53.44% reduction, while F1 had the least effect (25.82%). The enhanced activity in F3 could be attributed to a more optimized concentration of active phytochemicals and possible synergistic effects of the bioactive compounds identified in the GC-MS analysis.

Physical stability evaluation of the mouthwash formulations was conducted over a period of 30 days. All formulations retained consistent color, odor, and taste throughout the study period. The pH remained stable in the slightly acidic range (6-7), which is considered favorable for oral formulations as it discourages microbial growth without being overly erosive to enamel. Slight sedimentation observed in all formulations at Day 30 was re-dispersible and did not affect the overall homogeneity. Centrifugation revealed no signs of phase separation or turbidity, suggesting stable formulation. Temperature and viscosity parameters remained unchanged, further supporting the physical robustness of the preparations.

CONCLUSION:

An Herbal mouthwash based on *Terminalia chebula* was developed and evaluated in this study. The phytochemical screening

confirmed the presence of key bioactive constituents such as saponins, flavonoids, phenolic compounds, and tannins, known for their antimicrobial and antioxidant properties. GC-MS analysis revealed a complex chemical profile dominated by antioxidant-rich compounds, suggesting potential for both therapeutic and preservative effects.

Among the three formulations, F3 exhibited the highest antimicrobial activity against *Streptococcus mutans*, with a 98.88% microbial reduction, highlighting the effectiveness of higher concentrations of *T. chebula* extract. Physical stability studies demonstrated that all formulations maintained consistent physicochemical characteristics over 30 days, with no signs of degradation, phase separation, or significant changes in pH, viscosity, or sensory attributes.

Overall, the study establishes *Terminalia chebula*-based mouthwash as a promising natural alternative to conventional chemical mouthwashes. It offers significant antimicrobial efficacy, good stability, and potential for long-term use in maintaining oral hygiene, particularly for individuals seeking herbal and side-effect-free oral care solutions. Additional clinical research is required to validate its efficacy in diverse clinical settings.

CONFLICT OF INTEREST:

The authors have no conflicts of interest**.**

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