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Anticancer Activity and Phytochemical Analysis of Red Algae, *Gelidiella acerosa* Collected from the Gulf of Mannar.

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Abstract:

Cancer is a major health problem around the world, and there are still no fully effective treatments. Marine macroalgae, sometimes known as seaweeds, contain a variety of bioactive secondary metabolites that have antioxidant, anti-inflammatory, and anticancer properties. Phytochemicals generated from natural products have been identified as a new generation of anticancer medicines that may reduce the risk of cancer while posing minimal damage to patients.. This study explores the phytochemical constituents, GC-MS and anticancer properties of the ***Gelidiella acerosa***. To evaluate the phyto components , GC-MS and Anti-tumor activity against skin cancer cell lines (A431) was assessed by 3-(4,5-Dimethylthiazol-2-cyl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Phytochemical investigations suggests that the ***Gelidiella acerosa*** showed the presence of phytochemicals like alkaloids, steroids, flavonoids and terpenoids which may contribute to its biological activities.. GC/MS analysis revealed the presence of 15 compounds , Nonadecane as the

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active antitumor constituent of ***Gelidiella acerosa.*** The findings show that the ***Gelidiella acerosa*** investigated here has a number of unique and novel ingredients, as well as active potent antitumor chemical constituents, and that it can be used as a potential antioxidant and anticancer agent for future uses in pharmaceutical and medical industries.

Keywords:

Antioxidant, Anticancer ,***Gelidiella acerosa***, Nonadecane and GC-MS

Introduction:

Cancer remains a major health problem in worldwide. It has been suggested as an alternate strategy to develop anticancer medications using natural substances derived from marine life.**(1)** It has great potential for the development of novel anti-cancer drugs with high biological activity and distinctive chemical composition. Over the last few decades, pharmaceutical companies and academic institutions have made significant efforts in deriving and identifying new marine products from marine organisms, with more than 3000 new anti-cancer compounds (2,3) Of particular interest are the products derived from seaweeds with anti-cancer potential in natural marine products.

Macroalgae are a group of autotrophic, holophytic and complex community, living in the marine environment. . It contains a different type of pigments. Chlorophyta-green algae contains chlorophyll pigment, Phaeophyta-brown algae contains fucoxanthin, and Rhodophyta-red algae contains phycoerythrin and phycocyanin. (4,5) Red seaweeds are well-known for their biological activities due to enrichment of phenolic residues. During the last decades, research has demonstrated that those unique compounds express beneficial properties for human health. Each compound has peculiar properties (e.g., anti cancer antioxidant, antimicrobial, antiviral activities, etc.) that can be exploited to enhance human health.(6) In recent years, natural compounds extracted from marine algae have been proposed as effective in inhibiting tumor growth, adhesion, invasion, and migration (7).

Very few works are carried out on the seaweed ***Gelidiella acerosa*** from the region of Gulf of Mannar, Rameswaram, India. Therefore, the present study was subjected to assess the phytocompounds, biological activity and anticancer of ***Gelidiella acerosa***.

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The present study was performed with marine seaweed ***Gelidiella acerosa*** red algae. The study was performed with the following objectives: (1) To investigate the preliminary phytochemical constituents present in the seaweeds 2) To identify and characterization using GC-MS and FT- IR (3) To evaluate the anticancer activity.

Materials and Methods

Collection of seaweed

The mature green seaweed of the ***Gelidiella acerosa*** species, collected from the Pamban coast (Gulf of Mannar), Ramanathapuram, Tamil Nadu, India (9.2798° N, 79.2291° E), has been taxonomically authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The species is well-developed and holds significant value.

Preparation and extraction of seaweed

In order to obtain a high-quality seaweed solvent extract, the seaweed samples were initially rinsed with seawater to remove any debris, sand particulate, epiphyte, as well as extraneous substances and it was transferred into sterile bags containing water, brought to the laboratory. Further, it was washed with running tap water, followed by distilled water later the seaweed was dried in a dark state and powdered with a milling machine. The Soxhlet apparatus was then used to extract the sample using various solvents (Methanol, Chloroform, Ethyl Acetate, Hexane, and Aqueous). The thimble containing the seaweed sample was carefully placed in the extractor chamber, and the respective solvent was poured at a 1:10 ratio in a reservoir round bottom flask (60°C) in a heating mantle. We ran 15 refluxes on each sample to get the good quality solvent extract. Finally, the solvent extract was then condensed in a low temperature, vacuum-filled rotary evaporator, resulting in a precipitate which was collected into a glass jar at a temperature of -20°C and stored for further analysis (8).

Qualitative phytochemical analysis of *Gelidiella acerosa*

Peach and Tracey (1995) and Raaman (2006). *et.al* .

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The phytochemical screening of ive solvent extracts was conducted using the standard methods of Peach and Tracey (1995) and Raaman (2006).The qualitative analysis revealed the presence of phytoconstituents such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, and proteins, indicating the medicinal potential of the seaweed species (9)(10).

GC-MS ANALYSIS

Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometry (MS) detector. 5 ml of sample was evaporated to dryness and reconstituted into 2 ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2010 [SHIMADZU] instrument with Db 30.0 column (0.25 µm diameter × 0.25 µm thickness). The oven temperature was programmed from 70°C (isothermal for 5 minutes), with an increase of 10°C / min, to 200°C, then 5°C / min to 280°C, ending with a 35 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; scan interval of 0.5 seconds and scan range from 40–1000 m / z. Helium was used as the carrier gas at 99.99 % pressure with flow 1.0 ml / min and electronic pressure control on. Samples were dissolved in ethanol and injected automatically.

Analytical condition

The injection temperature at 240°C, the interface temperature at 240°C and ion source temperature at 70°C were determined. The injection was performed in split less mode.

Identification of compounds (data analysis)

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operator in scan mode from 40 to 1000 m/z atomic mass units. The identification of the compounds in the GC-MS of sample was based on the database of National Institute Standard and Technology (NIST), which had standards for more than 62,000 compounds.

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The known components in the mass spectrum were compared with the spectra of known components stored in the NIST library, through which the name, molecular weight and structure of the compounds were disclosed. Identification based on the molecular weight, molecular formula, retention time and peak area %.

It is done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and traditional use as herbal medicine.

In-vitro* anticancer aactivity of *Gelidiella acerosa Mosmann, T.,*et.al* 1983.

Cell line

The skin cancer cell line (A 431) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

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MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. (14) (15)

Results and discussion:

Phytochemical analysis

The presence of secondary metabolites observed in three seaweeds namely *Geidiella acerosa*, In the 42 preliminary phytochemical analysis using different solvents system.

The results of the phytochemical screening of seaweed extracts were given in table 1. The phytochemicals present in seaweed extracts were phenol, flavonoid, carbohydrate and reducing sugars, protein and aminoacids, saponin and steroid. Tannins, terpenoids, saponin and glycosides showed varied distribution in different extracts. the chloroform extract of Geidiella acerosa.In case of hexane extract, Geidiella acerosa found to have steroids, terpenoids and alkaloids.

Similarly methanolic extract of Geidiella acerosa, showed significant occurrence of steroids, terpenoids, alkaloids, saponins and phenols but two compounds like flavonoids and tannins was completely absent.However inaqueous crude extract of Geidiella acerosa contains presence of flavonoids, tannins, steroids, terpenoids, alkaloids and phenols were commonly observed but coumarins and saponins was absent. Likewise, flavonoids, tannins, steroids and alkaloids were seen in aqueous extract of but tannins was entirely absent in all solvent methods respectively.

The identified compound has the property of cytostatic, antiviral, anthelmintic, antifungal and antibacterial properties [16).. Similarly, the phytoconstituents of *P. hornemannii, H. floresii, C. compressa* and *G. acerosa* has been reported in many research works.(17)

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Table 1 Qualitative analysis of phytoconstituents present in different solvent extracts of *Gelidiella acerosa*

Phytochemicals	Aqueous	Hexane	Methanol	Ethyl acetate	Chloroform
Alkaloids	++	++	+++	++	++
Phenols	++	++	+++	+++	+
Flavonoids	++	+++	++	++	+
Tannins	-	-	-	-	-
Saponins	+	++	+	-	-
Terpenoids	+	+	++	+	++
Steroids	++	++	++	++	++
Carbohydrates	++	++	++	-	+
Glycosides	++	+	++	-	+
Amino acids	+	++	+	-	+
Proteins	++	++	+	++	+

+ → present in small concentration; ++ → present in moderately high concentration;

+++ → present in very high concentration;--→ absent.

Gas Chromatography–Mass Spectrometry (GC–MS) of the seaweeds

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GC-MS analysis technique can be effectively applied to determine the phytochemical profile of the algal extracts. Various reports have presented the chromatographic analysis of seaweeds revealing their phytochemical potential (Jainab *et al.*, 2019; Kannan & Priya, 2019). It was found that many of the identified compounds were reported to possess antimicrobial, antioxidant, anti-inflammatory, antitumor, cancer preventive properties. Fatty acids are reported to exhibit antibacterial, anti-inflammatory and antifungal activity (Uma *et al.*, 2017).

The present analysis ***Gelidiella acerosa*** extract showed the presence of **19 bioactive compounds** in the chromatogram (Fig. 1). The compounds were identified as Tetraethyl silicate, Diethoxymethyl acetate, 4H-Pyran-4-one, 2(1H)-Naphthalenone, Ionone, 2-Methoxy-4-vinylphenol, Diethyl Phthalate, 2-Propenoic acid, Ingol 12-acetate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, Phytol, 9,12,15-Octadecatrienoic acid, Vitamin E, Stigmasterol, Chondrillasterol, Stigmast-7-en-3-ol, 9,19-Cyclolanost-24-en-3-ol, (3.beta.) and 9,19-Cyclolanostan-3-ol in the retention time range of RT 4.417 to 40.709. These compounds are considered as rich phytochemicals with wide range of activities like antimicrobial, antioxidant, anti-cancer, anti-inflammatory, anti-allergic, anti-androgenic, anti-asthmatic, anti-diabetic activities in Table 2

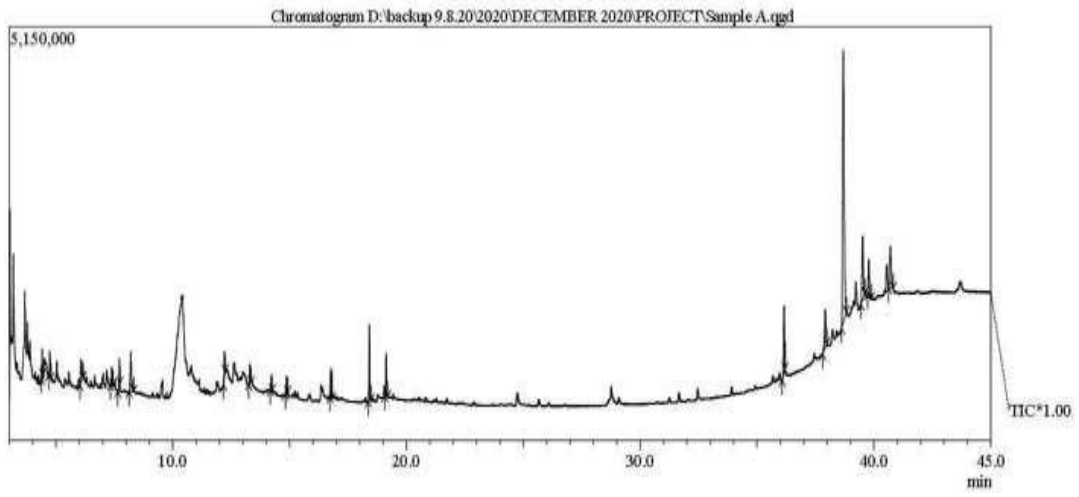

Fig.1 GC-MS chromatogram of *Gelidiella acerosa*

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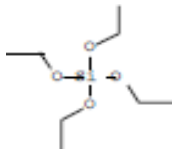
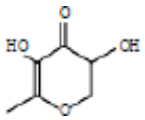
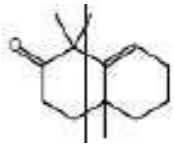
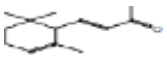
Table 2- Phytocomponents identified by GC-MS Analysis of *Gelidiella acerosa*

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak (Area %)	Molecular Structure	Reported Bioactivity
1.	4.417	Tetraethyl silicate	$C_8H_{20}O_4Si$	208	1.61		Antimicrobial activity
2.	4.738	Diethoxy methyl acetate	$C_7H_{14}O_4$	162	1.91		Antimycobacterial activity
3.	6.078	4H-Pyran-4-one	$C_6H_8O_4$	144	2.94		Antimicrobial activity, antibiofilm activity
4.	7.392	2(1H)-Naphthalenone	$C_{13}H_{20}O$	192	1.18		Anticancer activity
5.	7.718	Ionone	$C_{13}H_{20}O$	192	1.62		Antiproliferative, anticancer, anti metastatic activity

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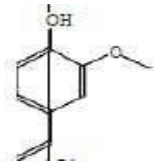
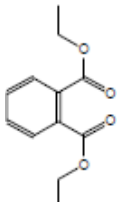
6.	8.204	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	4.52		Antiinflammatory
7.	12.219	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	1.61		Antiandrogenic activity
8.	13.307	2-Propenoic acid	$C_{16}H_{30}O_2$	254	1.30		Antiallergy activity
9.	14.214	Ingol 12-acetate	$C_{22}H_{32}O_7$	408	0.89		Antioxidant and anti microbial activity
10.	14.854	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.72		Antiinflammatory, antioxidant activity
11.	16.771	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	2.05		
12.	18.412	Phytol	$C_{20}H_{40}O$	296	5.59		Antihyperalgesic, Anti-inflammatory, and Antiarthritic activity

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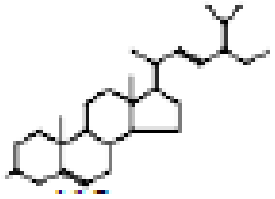
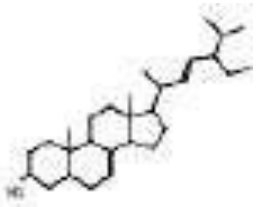
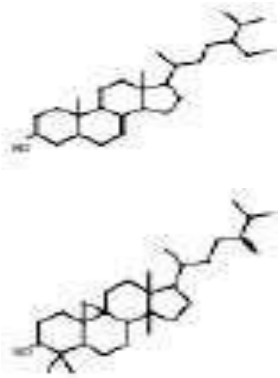
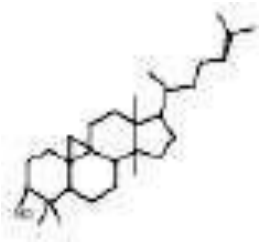
13.	19.135	9,12,15-Octadecatrienoic acid	$C_{20}H_{34}O_2$	306	3.57		Antiviral, anti inflammatory, antioxidant activity
14.	36.158	Vitamin E	$C_{29}H_{50}O_2$	430	6.72		Antioxidant activity
15.	37.923	Stigmasterol	$C_{29}H_{48}O$	412	4.88		Antimicrobial activity
16.	38.697	Chondrillasterol	$C_{29}H_{48}O$	412	40.42		Antimicrobial, anti ulcerogenic effect
17.	39.524	Stigmast-7-en-3-ol	$C_{29}H_{50}O$	414	8.09		Antimicrobial activity
18.	39.785	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$	426	4.33		Antiasthmatic, anti diabetic, antioxidant activities
19.	40.709	9,19-Cyclolanostan-3-ol	$C_{31}H_{52}O$	440	6.04		Antioxidant and anti

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							microbial activity

Invitro anti-cancer activity - MTT assay

MTT assay was performed to investigate the anticancer activity of the algal extracts on the human skin cancer cell line - A431. Agal samples demonstrated cytotoxic activity in a concentration dependent manner. The methanol extract of ***Gelidiella acerosa*** showed maximum cytotoxicity at **300µg/mL** with **72.37%** of the cancer cell inhibition, and the 50% threshold of cell inhibition was observed at a low concentration of **150 µg/mL** with **60 %** inhibition (Table 3 Fig, 2.)**.** It was observed that with increase in concentration the percentage of cytotoxicity increased from **17.89 %** at **33.26 µg/mL** to the highest **72.37%.** The viability of cells with ***Gelidiella acerosa*** extracts were visualized by cell line imaging at different concentrations (Fig. 3). The IC_{50} value of the extracts were obtained as **79.09 µg/ml** indicating the efficacy of the extracts.

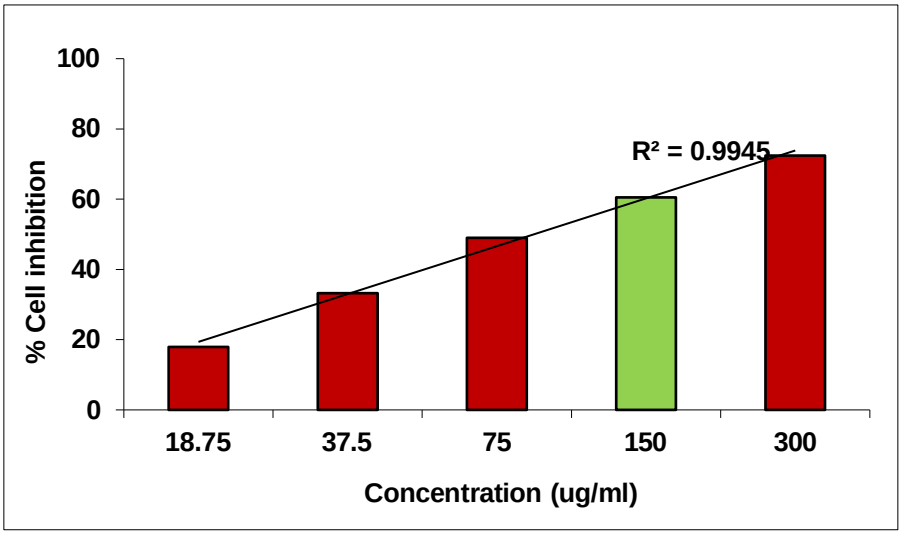

Fig 2 MTT assay on A431 cell line

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Table 3 MTT assay *Gelidiella acerosa* on A431 cell line

S.No	Concentration (µg/ml)	Absorbance (O.D)	% of cell inhibition
1	18.75	0.422	17.89
2	37.5	0.343	33.26
3	75	0.262	49.02
4	150	0.203	60.5
5	300	0.142	72.37
6	Control	0.514	-

Control

18.75 µg

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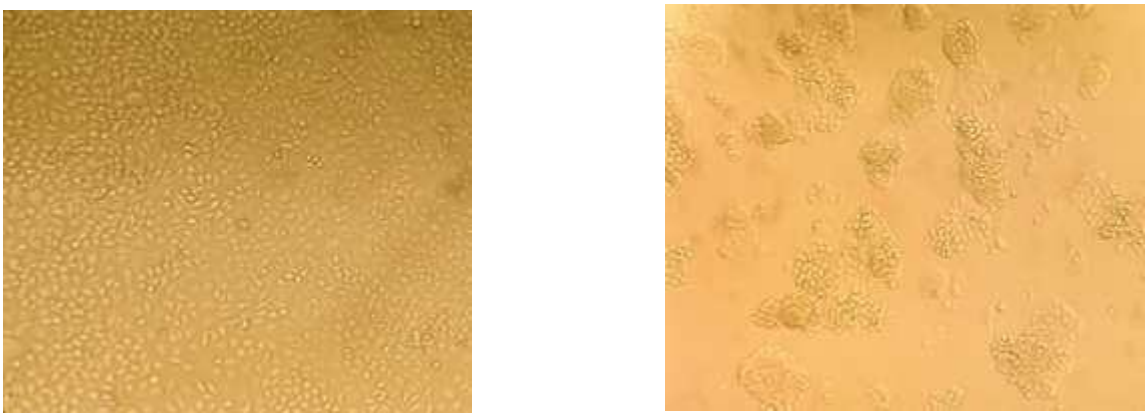

37.5 µg **75 µg**

150 µg **300 µg**

Fig. 4.35. A-431 cell line with different concentration of *Gelidiella acerosa*

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