A study of In-vitro antioxidant and cytotoxic activity of Haloplegma and Halymania seaweeds against Human A549 cell line.

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ABSTRACT

Screening of compound with potent biological activity from natural sources is need of the hour. Many pharmaceutical companies are focusing on the seaweeds to isolate potent compounds. The present study also focuses on screening the In-vitro antioxidant and cytotoxic activity of see weeds *Haloplegma duperreyi montagne* and *Halymenia dilatata zanadarini***.** In this study we used methanolic extracts of seaweeds. Methanolic extraction process was carried out using Soxhlet apparatus. The preliminary phytochemical screening was done. In-vitro antioxidant activity was studied using DPPH and ABTS radical scavenging activity. Cytotoxicity against lung cancer cell line was studied using A549 cell line. Both the extract showed best antioxidant activity and cytotoxic activity against A549 cell line. Extract 1 showed 56.16% cell death and extract 2 showed 62.08% of cell death. Both the sea weeds showed cytotoxic activity against lung cancer cell line A549. Further studies have to be carried out to find the mechanism of the cytotoxic activity.

KEY WORDS Sea weed; Antioxidant activity,Haloplegma, Cytotoxic activity, MTT,Lung cancer.

1 Introduction

Drug discovery from natural compounds play an insignificant role in the treating many disorders such as Vincristine and Paclitaxon for Cancer, Statins for cardiovascular disease, and fingolimod for treating multiple sclerosis (Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. et al. 2021). In recent years, the search for the natural products-based drugs were mainly for the five major dreadful disease including cancer, AIDS, heart disease, diabetes and pulmonary disorders (Anand, N. 2016). From the ancient period, nutritional compounds have been used to prevent cancer development. Proper intake of fresh vegetables and fruits is shown to reduce the risk of cancer onset (Gullett et al.2010). Various lead compounds from

natural products including Genistein, Lycopene, Brassinin, Sulforaphane, Indole-3-carbinol, Camptothecins, Combretastatins, Homoharringtonine, Resveratrol, Halichondrin B and Eribulin are in preclinical or clinical trials for cancer chemoprevention (Gullett NP et al.2010 &Cragg GM, Pezzuto JM 2016). Exploring for lead compounds from marine organisms apart from terrestrial regions also leads to the development of new compounds with unique cancer prevention mechanisms (Cragg GM, Pezzuto JM 2016).

Among marine organisms, pharmaceutical companies are looking for the products from sea weeds to be used in medical and biomedical research (Smit, A. J. 2004). Seaweeds are extensively scattered in all temperatures and are mainly responsible for maintaining the biodiversity in the ecosystems of aquatic regions. The popular ingredient in the East Asian food is seaweedor macroalgae. Sea weeds are macroscopic in nature throughout their lifetime,multicellular and photoautotrophic organisms and their inhabitat is mostly in the ocean or brakish water (Cotas J, Pacheco D, et al.2021). Seaweeds are nutritious and have a high quantity of proteins, minerals, and low quantity of lipids (Lange KW et al. 2015). Among the various phytochemicals in the seaweeds, polyphenols and sulphated polysaccharides are highly present in the seaweeds and possess many pharmacological properties (Sith Ranga Boopathy N, et al.2010). Many compounds from seaweeds showed antitumor activity by various mechanisms including impeding the growth of cells, migration of cells, cell cycle arrest, and induceing the apoptosis in the cancer cells (Qin, Y. (Ed.).2018). In the present study two seaweeds Haloplegma and Halymenia were chosen to study for their anti-cancer activity. *Haloplegma duperreyi Montagne* belongs to the family of Ceramiaceae. The color of the thallus varies from red -brown to grey-red. This seaweed is widely distributed in West Indies, tropical and subtropical Indian Ocean and Japan.*Halymenia dilatata zanadarini*is ared algaethat is known to contain various secondary metabolites and reported to have many biological properties including antimicrobial, antioxidant and anticoagulant activities (Deepak P, etal.2019). In the present study cytotoxic activity of the two seaweeds was studied in the A549, lung cancer cell line.

2 Materials and Methods

2.1 Procurement of seaweed

The 2 seaweed species *(Haloplegmaduperreyi montagne &Halymenia dilatata zanadarini*)were collected from Mandapam, Ramanathapuram district - Coordinates: 9.28°N 79.12°E. The collected sample was washed thrice in fresh water, rinsed in double distilled water, shade dried and blended into fine powder.

2.2 Extraction of seaweeds

The 100 gms of the grounded seaweed was packed in the Soxhlet apparatus and extracted using 500 ml of methanol for 72 hours. After the time period, the methanolic extract was collected and condensed in the rotary evaporator. The extract was dried and stored at -20°c until further use.

2.3Phytochemical Screening

Various phytoconstituents like polyphenols, flavonoids, quercetin, and polysaccharides were qualitatively screened as per the standard protocol (Harborne, J.B. 1973).

2.4 In-vitro antioxidant assay

2.4.1 DPPH Radical Scavenging activity

150 µM of DPPH was prepared in methanol. To 0.9 ml of the extract 0.1 ml of DPPH was added and incubated at dark for 30 minutes. After the incubation period, the absorbance was measured at 517 nano meter (nm) (Vijayamuthuramalingam UD2017). DPPH without the extract will be considered a control blank. Ascorbic acid was used as standard. The percentage of incubation was calculated using

% Of inhibition = (Absorbance of control-absorbance of the test sample)/Absorbance of control *100.

2.4.2 ABTS Radical Scavenging activity

ABTS (7.4mM) should be incubated with Potassium persulfate (2.6mM) for 16 hours to form the free radical 2,2-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) (ABTS•+). After 16 hours of incubation, the absorbance was measured at 734 nm. The ABTS•+ solution has to be diluted with methanol until the absorbance reaches 0.706±0.001 at 734 nm. To 100µl of the samples at different concentrations, 3.9ml of ABTS•+ was added and the absorbance was measured at 734 nm after six minutes (Vijayamuthuramalingam UD 2017). Ascorbic acid was used as the standard and the percentage of inhibition was calculated by using the formula

%Of inhibition = (Abs of control -Abs of test) /Abs of control x100.

25Cytotoxic activity of extracts

2.5.1 Maintenance of Cell lines

A549 cancer cell line and 3T3 a normal cell line was procured from National Centre for Cell Sciences (NCCS). The cells were maintained in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 5% Fetal Bovine Serum and antibiotics at 37°c at 5% Co2.

2.5.2 Cytotoxicity assay using MTT assay

Briefly5*10^3 cells per well were seeded into 96 - well plate and incubated at 37°c. After confluency, A549 and 3T3 cell line were treated with different concentration with bothextracts. After 24 hours of incubation, media was removed and 20 µl of MTT was added and incubated. After 2 hours the formazon crystals were dissolved by adding 100 µl of DMSO. The absorbance was measured at 570 nm (Arockiasamy S, Kuppuswamy, VR 2012).

3Results andDiscussion

3.1Phytochemical screening of the extracts

Both themethanolic extracts containreducing sugars and proteins. Extract 1 contains polyphenols and tannins when compared to extract 2. Flavones was absent in extract 2 (Table 1). Saponins and quinones were present in both the extract.

S. N	Phytoconstituents	Extract 1	Extract 2
1	Polyphenols	++++	+++

2	Reducing sugars	+++	+++
3	Flavones	++	-
4	Saponins	++	+
5	Alkaloids	+	+
6	Quinones	+	+
7	Proteins	+++	+++
8	Tannins	+++	+

Extract 1: *Haloplegma duperreyi montagne***; Extract 2:** *Halymenia dilatata zanadarini*

3.2 In-vitro antioxidant activity

In-vitro antioxidant activity of the two extracts was performed using DPPH and ABTS. Both methanolic extracts showed good scavenging activity of DPPH with the IC50 value of 0.49 mg/ml and 6.45 mg/ml for extract 1 and extract 2 respectively. Extract 1 at the concentration of 1 mg/ml showed 72% inhibition and the extract 2 showed 67.5% inhibition at 1mg/ml concentration.

In ABTS scavenging activity, Extract 2 showed best IC50 value of 0.075 mg/ml when compared with extract 1 with the IC50 value of 0.432 mg/ml. Extract 2 showed 78% inhibition at the concentration of 1 mg/ml and the extract 1 showed 65.5% inhibition at the concentration of 1 mg/ml.

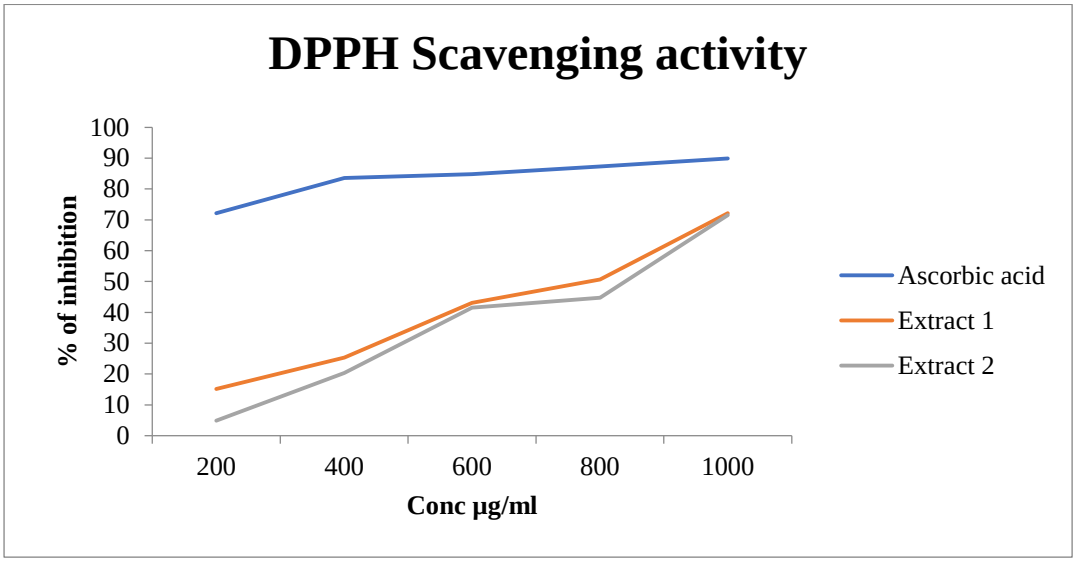

Fig 1: DPPH radical scavenging activity of two sea weed extract. Ascorbic acid was used as the standard. Extract 1 and 2 as described in table 1.

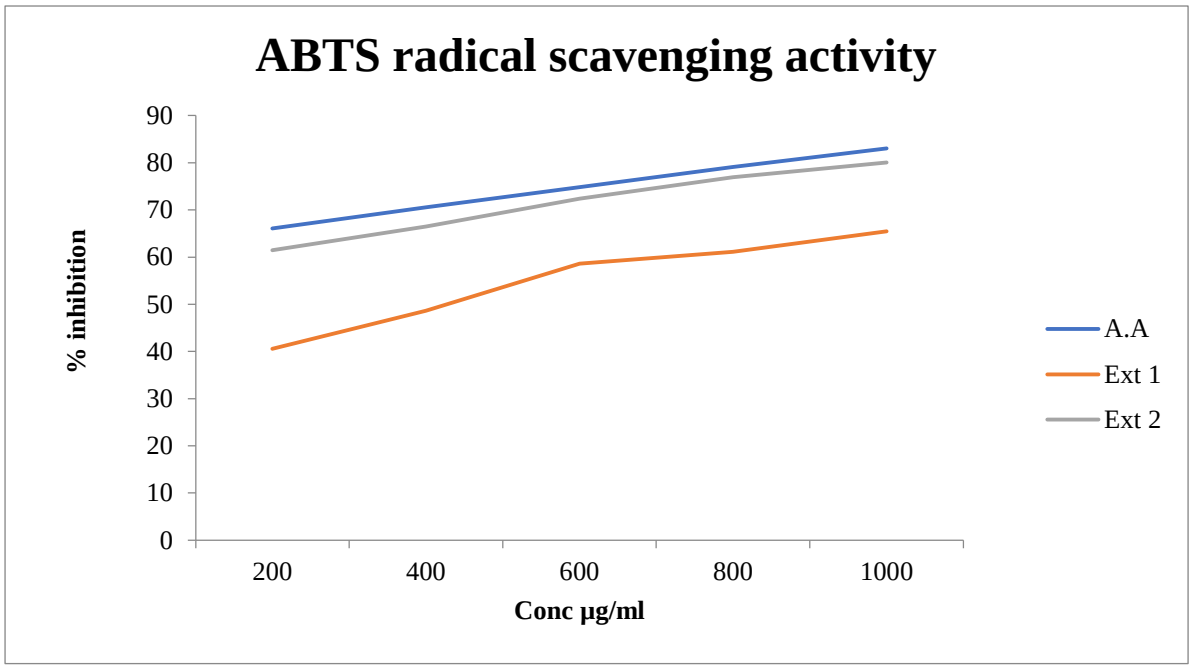

Fig 2: ABTS.+ radical scavenging activity of two seaweed extracts. Ascorbic acid was used as the standard. Extract 1 and 2 as described in table 1.

3.3 Cytotoxicity of seaweeds against A549 cancer cell line:

To evaluate the cytotoxicity effect of seaweed extracts against the A549 cancer cell line, an MTT assay was performed. Extract 1 showed 56.16% of cell death at the concentration of 1000 µg/ml and extract 2 showed 62.08% of cell death at the concentration of 1000 µg/ml (Fig 3). Phase contrast images of control cells showed distinct cell morphology. In treated cells reduced cell densitywas observed and the morphology of the cells was changed.

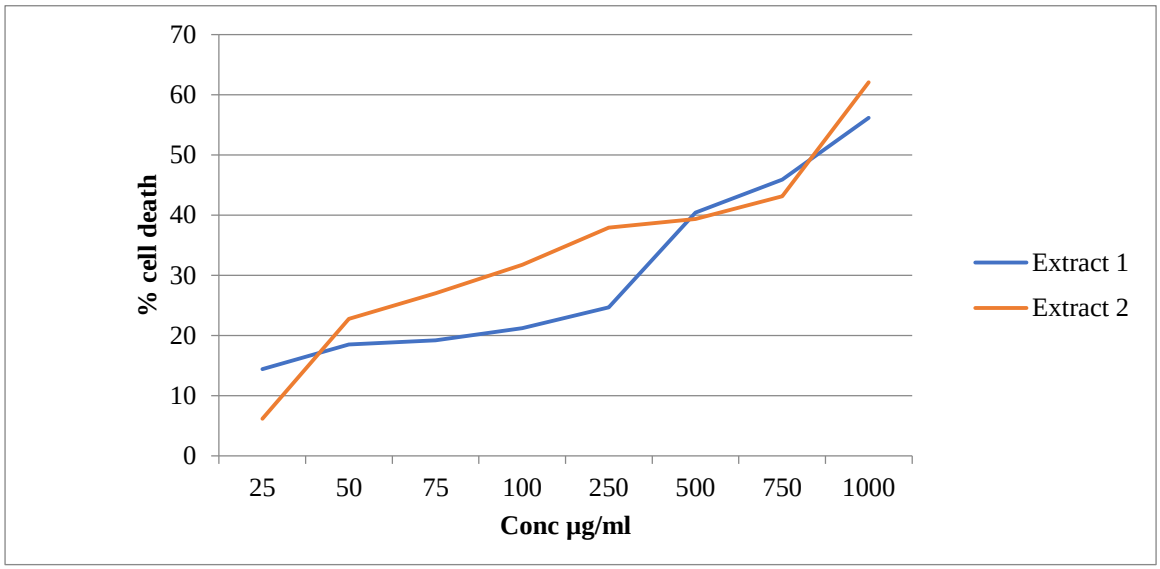

Fig 3: MTT assay showed percentage of cell death induced by seaweed extract at various concentration in A549 cell line. Extract 1 and 2 as described in table 1.

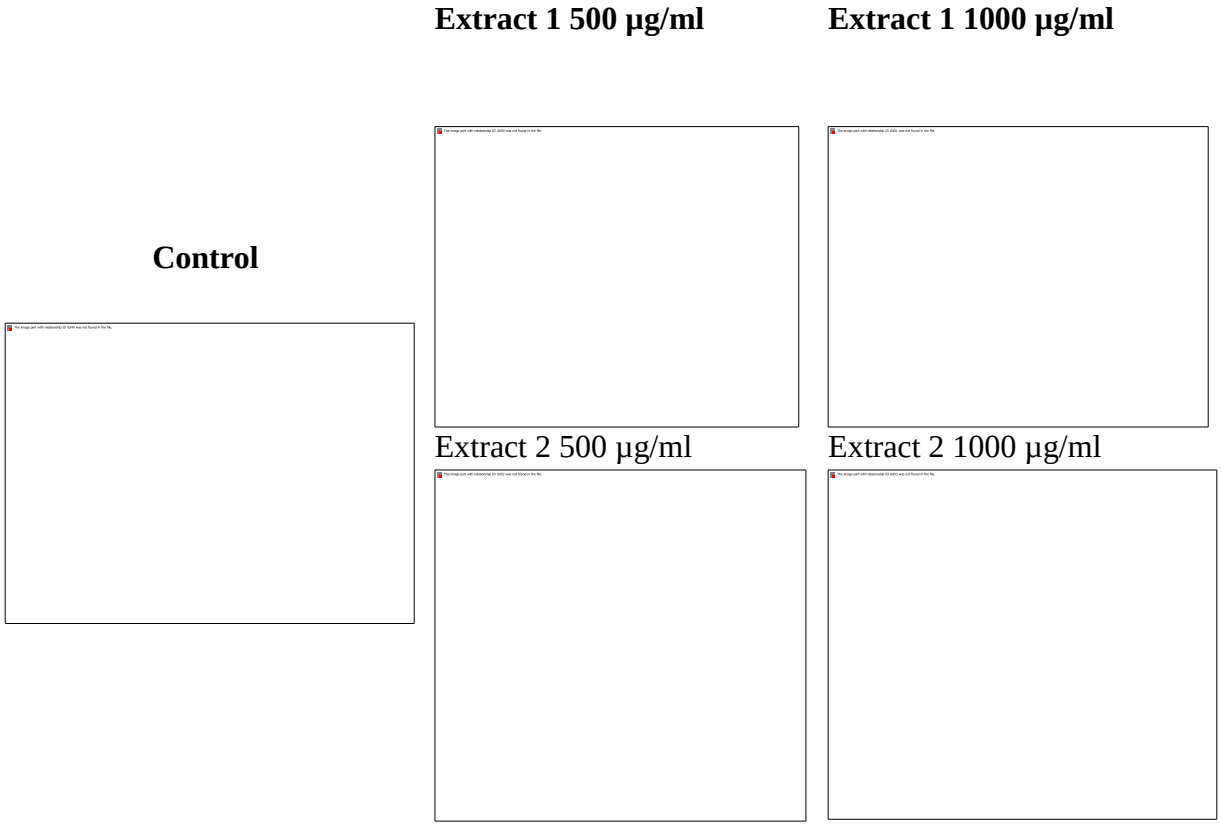

Fig 4: Phase contrast image ofA549cell line. Control cells showed distinct morphology and were healthy. Treated cells showed reduced cell density with distorted cell morphology.

3.4 Cytotoxic effect in normal cell line

To evaluate the cytotoxic effect in the normal cell line, the 3T3-L1 mouse fibroblast cell line was used. Various concentration of the extract was used and the MTT assay showed cells was not toxic to the 3T3 L1 cell line (Fig 5). 80% of viable cells were observed in extract treated cells.

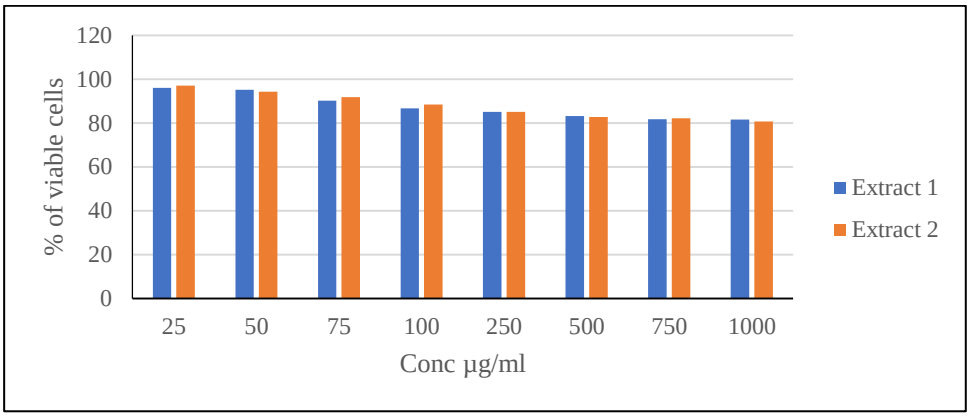

Fig 5: Percentage of viable cells in the 3T3-L1 cell line.

In the marine environment sea weeds are rich in bioactive compounds and many studies are carried out to screen the potential chemotherapeutic activity. In the present study, the methanolic extract of two sea weeds namely *Haloplegmaduperreyi montagne* and *Halymenia*

*dilatata zanadarini*was prepared. Since many studies showed methanolic extract is more active when compared to choloform and ethylacetate, methanolic extraction was carried out in the present study (El-Din SM, El-Ahwany AM 2016;Shyamala V, et al. 2014). The phytochemical screening reveals the presence of polyphenols and polysaccharides abundantly in the both extracts when compared with other phytochemicals. Phenolic compounds are highly present in the terrestrial plants and marine plants and these compounds are mainly responsible for their biological properties. Apart from polyphenols, polysaccharides were also highly present in the extract which was comparable with the previous study (Liu Z, Gao T, et al. 2019). Polysaccharides are mainly present in the fibrillar regions and intracellular space of the cell wall (Senthil Kumar K, et al.2013). Polysaccharides isolated from seaweeds are used for the commercial purposes including agar, alginic acid, laminarine, fucoidan etc. Due to the presence of polyphenols the extract showed antioxidant activity with scavenging DPPH and ABTS with agood IC50 value. Previous studies also showed similar results with good antioxidant activity in sea weeds (Shyamala V, et al. 2014). The antioxidant activity may be due to the presence of pigments and polyphenols.

Polyphenols is an important bioactive molecule present as secondary metabolite possess various health benefits. These polyphenolic compounds can act as an anticancer activity by various mechanisms. Modulation in the signalling pathways removes the cancer cells, inhibiting the cancer cell cycle mechanism, upregulating the apoptotic pathways are some of the mechanisms by which polyphenols possess the anticancer property (Bhosale PB, et al. 2020). Seaweeds rich in polyphenols possess the anticancer activity. Similarly, polysaccharides highly present in the sea weeds also have anticancer activity. The polysaccharides showed anti-cancer activity by activating the apoptotic pathways or it show modulate the immune system to target the cancer cells. The presence of these two phytoconstituents in the sea weed extract may contribute to the anti-cancer activity (Zhang F, et al.2017). Therefore, the present study also focuses on the cytotoxic effect of methanolic extract of two sea weeds against lung cancer cell line.

The prevalence of lung cancer and mortality due to lung cancer is very increasing in the recent years. Globally the lung cancer ranks first in for the mortality in men and the in women it ranks second for causing mortality after breast cancer (Xing DF, et al.2019). Many chemotherapeutic drugs are available on the market for treating the lung cancer but the side effects are very high. Thus, continuous research was carried out to screen some active compounds from the natural products to screen the potential drug with fewer side effects in treating cancer (Poofery J, et al.2020). Such an attempt was taken in the present study to screen for the cytotoxic activity of seaweeds against lung cancer cell lines.

The present study also screened for the cytotoxic activity of the extract against Lung cancer cell line A549. Dose dependent cytotoxicity was observed in the A549 cell line. A Previous study also observed a methanolic extract of *Gracilaria edulis*, a red algae showed dose dependent cytotoxic activity in the Breast cancer cell line (Hemasudha TS, et al. 2019). The present study showed that the extract is not toxic to the normal cells. To our knowledge it is the first repot to show the cytotoxic effects of *Haloplegmaduperreyi montagne* and *Halymenia dilatata zanadarini*in the cancer cells.

Conclusion

Searching for lead compound for anti-cancer activity is the need of the hour to combat the increased mortality due to cancer. The present study revealed *Haloplegma duperreyi montagne* and *Halymenia dilatata zanadarini* methanolic extract showed cytotoxic activity against the A549 cell line. The isolation of compound from the extract may lead to the development of lead compound for anticancer drugs.

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Conflicts of interest and financial disclosures

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